

Therapeutic strategies for human papillomavirus infection and associated cancers

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1. ABSTRACT

Human papillomavirus (HPV) infection is linked to development of cancer of cervix, vagina, vulva, penis, ano-genital and non-genital oro-pharyngeal sites. HPV being a sexually transmitted virus infects both genders equally but with higher chances of pathological outcome in women. In the absence of organized screening programs, women report HPV-infected lesions at relatively advanced stages where they are subjected to standard treatments that are not HPV-specific. HPV infection-driven lesions usually take 10–20 years for malignant progression and are preceded by well-characterized pre-cancer stages. Despite availability of window for pharmacological intervention, therapeutic that could eradicate HPV from infected lesions is currently lacking. A variety of experimental approaches have been made to address this lacuna and there has been significant progress in a number of lead molecules which are in different stages of clinical and pre-clinical development. Present review provides a brief overview of the magnitude of the problem and current status of research on promising lead molecules, formulations and therapeutic strategies that showed potential to translate to clinically-viable

HPV therapeutics to counteract this reproductive health challenge.

2. INTRODUCTION

Strong awareness drives around the globe against sexually transmitted infections and organized periodic cervical screenings using a simple cytology smear test devised by Papanicolaou have brought down cervical cancer from its topmost killer position to the fourth leading cause of cancer death in women (1, 2). An estimated 527,600 new cervical cancer cases, 265,700 deaths were reported worldwide in 2012 (3). With a due credit to the organized cervical screening, the incidence of invasive cervical cancer is now restricted significantly in the developed countries. In contrast, a disproportionately high disease burden, exceeding 90% of total, is contributed solely by the less developed countries. Cervical cancer, which is usually reported late and in advanced stage of the disease in most of the developing nations, represents a formidable reproductive health challenge (4). Clinico-epidemiological and molecular studies have

established causal link between persistent infection of human papillomavirus (HPV) with neoplastic growth of uterine cervix and other parts of the reproductive tract (5, 6). HPV-induced tumors can be either benign or malignant depending upon the genotype (s) of the infecting virus (7). These infection-induced tumors are frequently detected in cervix and to a lesser extent in vagina, vulva, penis, ano-genital and non-genital sites (8), though the non-cervical tumors are showing an increasing trend in last two decades (6, 9). The earliest evidence of sexual route of transmission of these cancers came with historical notions (10) and later supported by epidemiological studies that women with multiple sex partners or having spouses with multiple sexual contacts were at an increased risk of contracting these cancers (11, 12). Harald zur Hausen, a German virologist while researching on cancer of the cervix, discovered HPV as human carcinogen and a causative factor for onset and progression of genital neoplasia (13), for which he received the Nobel Prize in Physiology or Medicine 2008. With over 200 different genotypes having a varied genetic and oncogenic potential identified till date (14), this class of viruses is known to infect cutaneous and mucosal epithelium of both men and women worldwide. About 80% of women and likely the same number of men are estimated to get this infection sometime in their lifetime (15). Most of these incident infections are transient and clear spontaneously, more than 90% within one to two years; however, about 10% persist and can cause neoplastic transformation (16–19). Spontaneous clearance without any clinical intervention is common till 30–35 years of age; following which the clearance rate decreases with increasing age and decreasing immune status (16, 17). Discovery of infectious etiology of cancer catalyzed growth in understanding the basic biology and molecular diagnosis of the virus, formulation of cervical screening programs and development of various vaccines and therapeutics to control HPV infection in order to prevent or treat cervical and other HPV-associated neoplasias. Till date, prophylactic vaccines developed against the most prevalent oncogenic HPV that are given at an early adolescent age prevent incident infection and have become a clinical reality (20, 21). However, interventions against already infected lesions are still in different phases of pre-clinical clinical development and evaluation. Such therapeutic entities are, however, much needed in resource constraint under-developed nations that cannot support and implement nation-wide periodic screening or vaccination drives. As per estimates from World Health Organization's (WHO) Catala Institute of Oncology, 453 million women (female population aged ≥ 15 years) are at risk for cervical cancer, whereas only 3.1.% fraction of these women are covered by opportunistic cervical cancer screening (22). Present review is, therefore, aimed to provide a brief overview of the magnitude of the problem of HPV-associated malignancies with a particular emphasis

on cervical cancer, basic biology of the disease and status of research on lead molecules, formulations and therapeutic strategies that showed potential in recent time to translate to a clinically viable HPV therapeutic that could meet this reproductive health challenge.

3. SPECTRUM OF HPV-ASSOCIATED DISEASES AND MAGNITUDE OF THE PROBLEM

HPV infection is causally associated with cervical cancer; whereas in other genital cancers including anal, penile, vulva, and vaginal cancers, HPV infection may act as co-factor (6). Compared to cervical cancer, other HPV-associated ano-genital cancers are relatively less frequent (15, 23–25) (Figure 1). Apart from frank malignancies, HPV can cause various types of “genital” or “venereal” warts. Depending upon the frequency of association of a particular HPV genotype with malignant or benign lesion, HPVs are stratified as high-risk (HR) or low-risk (LR) types (5, 26). The established HR-HPV include HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 59; and LR-HPV include HPV6, 11, 40, 42, 43, 44, 54, 61, 72, and 81. Recently some of the types have been recognized as intermediate or probable high risk (pHR) which includes HPV26, 30, 34, 53, 66, 67, 68, 69, 70, 73, and 82. Among all these types HR-HPV type 16 and HPV18 in cervical cancer and LR-HPV type 6 and 11 in genital warts are the most common HPV types (15). HPV16 is the most virulent strain accounting for more than 50% overall disease burden and usually lead to squamous cell carcinoma, whereas HPV18 is primarily associated with a less common adenocarcinoma of the cervix. Population prevalence of HPV in asymptomatic, reproductively-active women is highly variable (15). The meta-analysis shows HPV positivity reported in this group range from as low as 0.1–100% in different studies. Such variations are ascribed to the test used and the population of women sampled. A median range of HPV prevalence is about 11.7% where HPV16 constitutes the highest proportion of 2.8% (15, 27). The proportions and spectrum of HPV types change with severity of the lesions (Figure 1).

Natural history of HPV infection and cervical cancer has revealed cytologically (low grade and high grade) and histopathologically (Cervical Intraepithelial Neoplasia or CIN1, CIN2, CIN3) well-defined pre-cancer stages that precede cervical cancer (28, 29) (Figure 2). The process of tumorigenic transformation from entry of the virus to invasive carcinoma takes 10–20 years and thus provides a desirable window period to treat this disease at an early stage. Early infection is primarily latent, subclinical, and opportunistic (30). Genital HPV infection is highly prevalent in sexually active young women. Furthermore, sexual contact with an HPV infected individual results in an increased probability of contracting the virus (11, 29, 31). Some

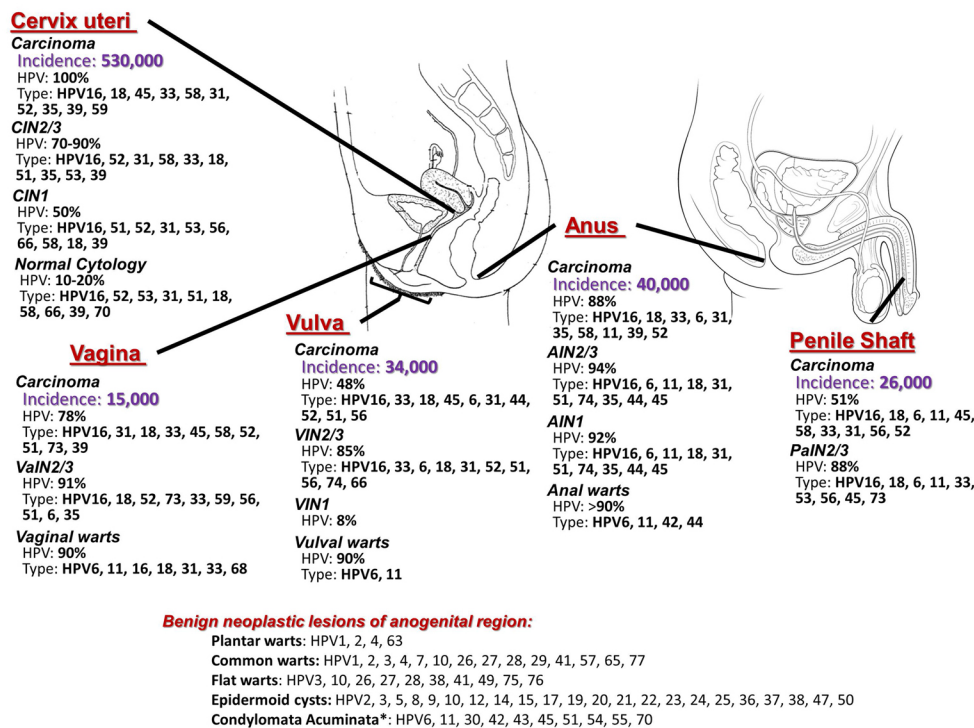


Figure 1. Incidence of HPV infection-associated benign, premalignant and malignant neoplasia of different ano-genital sites and commonly encountered HPV genotypes. *Condylomata acuminata commonly known as genital warts are found on genitalia i.e. shaft of penis in men and cervix, vulva and vagina in women, and in anus. Sequence of HPV genotypes indicated is in order of prevalence only in precancers and cancers. Abr: CIN – cervical intraepithelial neoplasia, VIN – vulval intraepithelial neoplasia, VaIN – vaginal intraepithelial neoplasia, AIN – anal intraepithelial neoplasia. Source: Incidence (25); genotype data (15, 24).

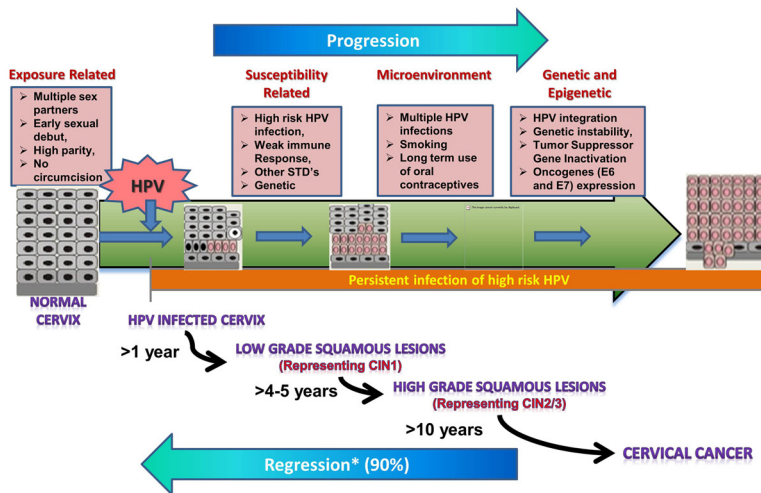


Figure 2. Natural history of HPV infection and stage-wise progression of cervical cancer. The boxes indicate risk factors potentially involved in progression of disease in particular stage. *The probability of regression of lesions decrease with progression of disease. Abr: CIN – cervical intraepithelial neoplasia.

additional risk factors include use of oral contraceptives, a history of sexually transmitted infections, smoking, and immunosuppression (32). Various factors depending upon their role in carcinogenic progression of the disease can be classified into exposure-related, host susceptibility-related, factors that affect tumor microenvironment, and somatic/epigenetic. Among factors that strongly influence HPV transmission and

pathogenicity are prevalence of different HPV types in a particular population, duration of infectivity, patterns of sexual contacts and pathogenic behavior of the infecting virus (28, 29) (Figure 3). Since HPV is a sexually transmitted virus primarily it is transmitted through heterosexual intercourse, however, oro-genital and anogenital transmission is also possible resulting in its presence in extra-genital sites that can occur due

Determinants of HPV Transmission and Pathogenesis

- **Population prevalence** (indicator of disease burden and the probability of encountering an infected partner)
- **Duration of infectivity** (host susceptibility and immunological status)
- **Patterns of sexual contacts** (sexual behavior)
- **Pathogenicity** (viral factors: benign vs oncogenic pathogenic or infective variants-strongly interacting with L1 cell surface, oncogenic E6/E7)

Figure 3. Determinants of HPV transmission and pathogenesis.

to varied sexual practices like oral and anal sex (33) (Figure 4). However, oral to oral transmission is still debatable. The basis of transmission is always a direct skin-to-skin contact. In the same corollary, condom provides protection but not 100% safe to prevent transmission. Interestingly, circumcision is found protective in reducing transmission thus highlighting its importance in prevention of viral transmission (34). Friction and micro-abrasions play a key role in exposure of dividing cells of the basal layer that host a productive viral infection (29). Spontaneous HPV clearance is another attribute of HPV infection among females. Majority of the cases regress spontaneously without any need of intervention. In a 12-year follow-up study of women with two positive tests for HPV16 (at baseline and 2 years later) the risk of CIN3+ lesions estimated was 47.4.% (95% confidence interval 34.9.–57.5.%). Several long-term natural history studies revealed a reduced risk of CIN3+ lesions in women who crossed the peak age of HPV acquisition (e.g., 30 years or older) who remain HPV-negative or could clear their HPV. CIN2 is considered as an intermediary step between CIN1 (HPV infection) to CIN3 (direct cancer precursor). Recent data have shown that regression rates of CIN1 among young women (defined as less than 25 years of age) are quite high (up to 90%) which fall as the lesion progresses (29, 30).

HPV is necessary but not sufficient to cause cervical cancer (35). Several other sexually transmitted infections such as *Candida*, *Trichomonas vaginalis*, *Chlamydia trachomatis*, and *HSV-2* may participate

as cofactors (35–37). It is likely that these infections result in localized chronic inflammation that provides a suitable niche for persistent HPV infection (Figure 5). A recent study evaluated association between bacterial vaginosis (BV) and inflammatory response (IR) with the severity of cervical neoplasia in HPV-infected women and have revealed vaginal infections irrespective of their nature (bacterial, fungal, yeast) increased the susceptibility, effects the clearance of HPV infection and eventually contributed towards HPV-induced cervical or genital carcinogenesis (38). These infections alter the microenvironment in the genital areas thereby causing severe inflammation, which en routes the development of cervical neoplasia. The key transcription factors that mediate inflammatory response, i.e. Nuclear Factor-kappa B (NF-κB), Signal Transducer and Activator of Transcription (STAT)-3, and Activator Protein (AP)-1 (39), are also important regulators of HPV infection (40–42) and have showed an aberrant expression and constitutive activity in cervical precancer and cancer tissues which increase with disease severity (43–45). These observations suggest potential utility of anti-inflammatory anti-cancer drugs in control of HPV infection and cervical cancer.

4. MOLECULAR CHARACTERISTICS AND INFECTION CYCLE OF HPV

HPV is an epitheliotropic, double stranded DNA virus and belongs to the family of papillomaviridae. HPV genome of about 8 kb consists of eight open

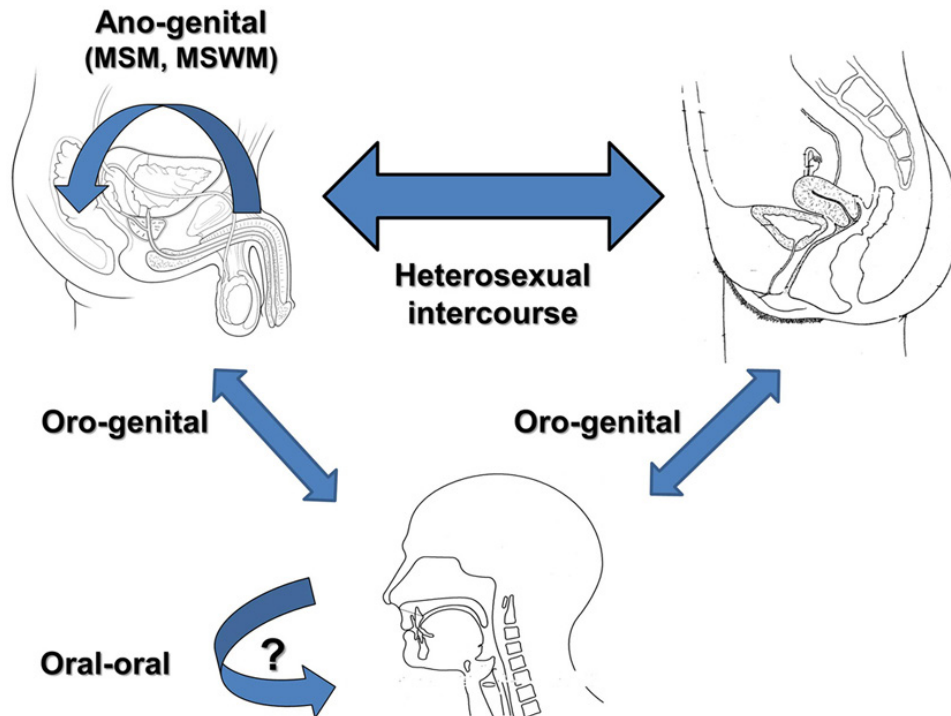


Figure 4. Routes and sites of HPV transmission. HPV being a sexually transmitted infection, heterosexual unprotected intercourse in the absence of condom may result in transfer of infection to the sexual partner. Unlike in case of HIV, condom is not an infallible method of protection from HPV specifically against cutaneous HPVs. There is no report for oral-oral transmission. Abr: MSM- men having sex with men; MSWM- men having sex with men and women.

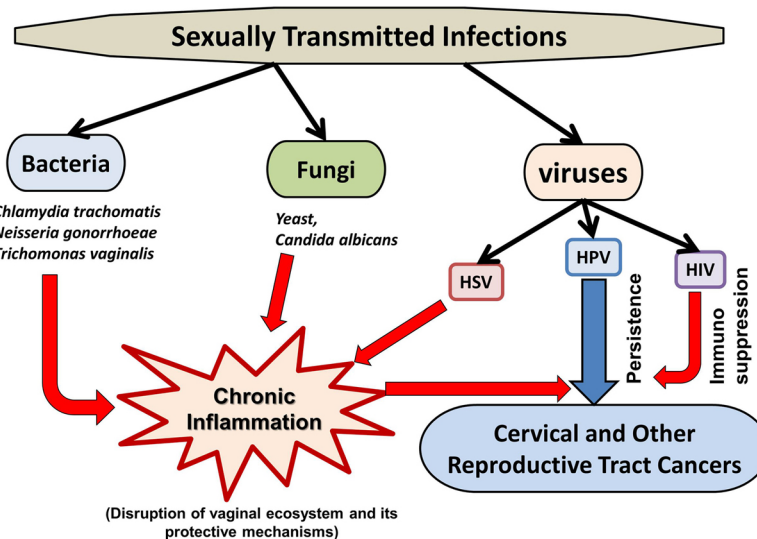


Figure 5. Sexually transmitted infections cooperate with HPV for its persistence by promoting inflammatory microenvironment leading to carcinogenic progression of cervical lesions. In contrast, HIV promotes HPV infection by compromising anti-viral immunity. Abr: HIV – human immunodeficiency virus, HPV – human papillomavirus; HSV – human simplex virus.

reading frames, encoding two structural proteins namely L1 and L2, which constitute the outer capsid of 55nm and 6 regulatory/functional proteins namely E1, E2, E4, E5, E6, E7 (46, 47). L1 protein has been characterized as highly immunogenic, and is found to be conserved across different HPV types. Due to

this fact, the protein has been well exploited for the development of different forms of DNA diagnostics and prophylactic vaccines, and in the serological assays. This protein is also important as it self assembles to form small virus like particles (VLP) which can elicit an active immune response. The L2 protein on the

other hand, constitutes the minor capsid protein. Other proteins encoded by HPV include E1 and E2. E1 has helicase activity and participate in viral replication, genome partitioning, and transcription. Proteins coded by E6, E7 and E5 HPV genes have key oncogenic role and disturb the normal cell cycle regulation leading to tumorigenic transformation. The oncogenic activities of E6 and E7 are well characterized and the proteins seem to have different host cell targets. The tumor suppressor protein p53, retinoblastoma protein (pRB) and epidermal growth factor receptor (EGFR) are targeted by E6, E7 and E5 respectively. Recent developments in bioinformatics suggest a more complex multiple interaction of viral oncoproteins with other key components of cellular machinery. These interactions collectively and synergistically promote continuous cell proliferation, DNA amplification, and impairment of DNA repair mechanisms leading to genome instability, and anti-apoptosis (47–49). E2 plays a strong regulatory role by promoting viral replication thereby promoting productive viral life cycle and preventing expression of E6 and E7 oncogenes from early promoter. Epigenetic silencing or inactivation of E2 due to integration of the virus DNA in host genome via E2 coding sequences results in loss of E2 expression which leads to (over) expression of E6 and E7 through an early promoter (p97). The viral genome can stably exist in both episomal and integrated forms. However, integration makes tumorigenic transformation an irreversible event. Viral gene expression is highly orchestrated that align with epithelial keratinization (49). Sequential expression of genes is controlled through a 800bp region named as upstream regulatory region (URR) that contains sequences for binding of (A) host transcription factors like NF- κ B, STAT3 and AP-1; and (B) HPV's transcription regulator E2 (40–42). Constitutive expression of E6 and E7 is a necessary prerequisite for growth and survival of cervical cancer cells as well as initiation and maintenance of malignant transformation (47). Recent investigations from our group demonstrated novel role of HPVE6 in maintenance of stemness and chemoresistance in cervical cancer cells (50, 51) by regulation of key signaling mediators like STAT3, Notch/hes and Gli (45, 50–53). These leads make HPV oncoproteins and their upstream regulatory mechanism a suitable target to design effective anti-HPV therapeutics.

5. CONVENTIONAL THERAPIES FOR MANAGEMENT OF BENIGN AND MALIGNANT CERVICAL LESIONS

Current primary and secondary prevention measures and clinical management of HPV infected lesions have been periodically reviewed (30, 54) and schematically summarized in Figure 6. Primary prevention of infection with high-risk HPV types

remains the most efficient and logistically feasible preventive measure for cervical cancer. Regulatory authorities initially approved two vaccines to prevent HPV infection i.e. Gardasil (HPV16, 18, 6, & 11) (20), Cervarix (HPV16 & 18) (21, 55, 56) and later in 2014 Gardasil 9 which targeted 5 additional HPV types (HPV31, 33, 45, 52, 58) apart from the earlier version of the vaccine (57). These HPV vaccines, if administered before sexual activity, can reduce the risk of infection of the HPV types targeted by the vaccine. Although the vaccines are safe and effective in preventing HPV16 and HPV18 persistent infections and associated CIN2/3 lesions in young women who had no HPV infection at the time of first vaccination (58, 59), neither of the two vaccines was able to clear existing HPV16/HPV18 infection, nor could prevent their progress to CIN2/3 (60, 61).

As a secondary prevention measure, screening of women by a regular gynaecological examination that include either a standard Pap smear test, visual inspection with acetic acid (VIA) or Lugol's iodine (VILI) is recommended. These are low cost screening tools that examine the presence of cytological or clinical lesions. Hybrid Capture II (HCII) and APTIMA are most popular United States Food and Drug administration (US-FDA) approved molecular tests based on detection of HPV DNA and RNA respectively with very high sensitivity, specificity and clinical relevance. Emerging techniques like OncoE6, CINTECP16 can detect HPV and its types. Cervista, first US-FDA-approved genotyping test can detect 14 high-risk HPV types (62) making it easier to know the HPV types involved in the disease. Detailed description of various other molecular tests and their targeted HPV genotypes has been made earlier (63). Nevertheless, HPV testing is gradually replacing cytology-based cervical screening owing to greater reassurance when the test is negative. Despite significant progress in both primary and secondary prevention strategies, the effective implementation of HPV vaccination and screening particularly in low resource settings remain a challenge.

Treatment modalities depend upon the type and location of manifestation i.e. genital warts, pre-cancer and finally cervical cancer. Genital warts can be treated by topical treatment (Imiquimod, Podofilox), interferons, cryotherapy surgical removal, trichloro acetic acid, carbon dioxide laser therapy [Reviewed in (64)]. Apart from topical treatments, all other interventions are provided by trained healthcare professionals. Interferon which by their nature are generic anti-viral agent, have been used for the treatment of laryngeal papillomas as well as cutaneous and anogenital warts (65). Partial and total remissions have been achieved with topical, intra-lesion and systemic administration of the interferon. While IFN- α

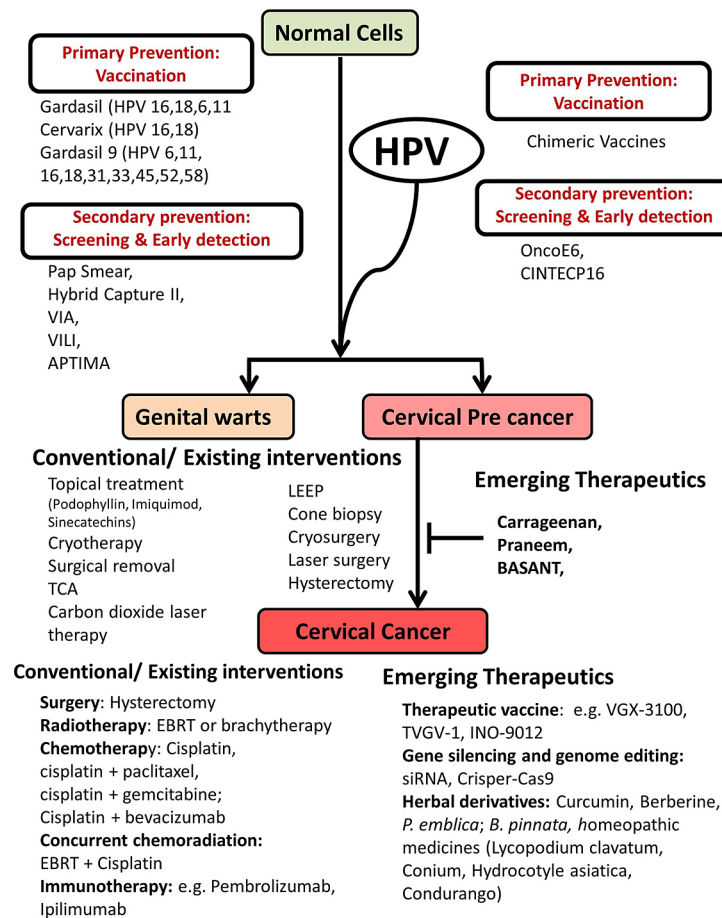


Figure 6. A bird's eye view of representative conventional and emerging modalities for prevention, diagnosis and treatment of benign and malignant cervical lesions. Abr: EBRT - External beam radiation, LEEP - loop electrosurgical excision procedure, TCA – trichloroacetic acid, VIA – visual inspection with acetic acid, VILI - visual inspection with Lugol's iodine.

is clinically approved for the treatment of genital warts and used as an essential part of treatment, it is generally not recommended, as the doses required for clinical effects are not tolerable by patients (66). In some reports, lack of efficacy of IFN- α therapy in recurrent, advanced cervical cancer was described. It shows minimal activity against advanced, recurrent cervical cancer (67).

Among patient applied therapies Imiquimod (Aldara) is a potent adjuvant for the topical treatment of HPV lesions and is an immunomodulator. Imiquimod works by stimulating the immune system to release cytokines, which are important in fighting viruses and destroying cancer cells. Several randomized controlled trials were conducted to estimate the efficacy of a treatment with imiquimod in patients with CIN 2–3 (68). Though, it is well tolerated, irritation usually develops on treated skin and it is sometimes not effective for all patients. Moreover, its long treatment time (that extends beyond 4 months) and cost is a major concern. Podophyllotoxin is another patient applied therapy which is widely available in a various formulations

apart from imiquimod. In general, podophyllotoxin is cheaper than imiquimod, whereas imiquimod 5% is associated with lower recurrence rates than podophyllotoxin (69). However, none of these methods are HPV-specific nor uniformly successful. Recurrence rates vary tremendously, from 5% to 65% depending on treatment modality (70).

Methods commonly used to treat precancerous cervical changes could be ablative and excisional, and include cryosurgery, loop electrosurgical excision procedure (LEEP), surgical conization and laser vaporization conisation which may require local or general anaesthesia. LEEP remains an important classic technique for obtaining an excellent diagnostic and therapeutic specimen. For settings where LEEP cannot be performed, cryotherapy is used as an alternative treatment for eligible VIA positive lesions. Cryotherapy is the only option available outside of surgical settings and more popular treatment with a widespread use in many countries because of its ease of use. However, a special attention and certainty is warranted in the diagnosis and visualization of the

lesion as the technique does not provide specimen for confirmatory histopathology (30).

Other techniques such as cone biopsy along with laser surgery can also be used. The treatment of choice in early cervical cancer is surgery which can either be radical or total hysterectomy depending on the location and size of the tumors (71). Radiotherapy is the treatment of choice for cervical cancer. For women with locally-advanced cervical cancer, external beam radiation therapy (EBRT) was the standard of care earlier. With advancements in radiotherapy, EBRT has been coupled with brachytherapy, or combined with brachytherapy and concurrent chemotherapy (72). Chemotherapy alone is generally ineffective for cervical cancer although it can complement other treatment regime in late stages (73). Chemotherapy when given with radiation can be an effective treatment of choice and largely accepted as the standard of care for patients with stage II to stage III cervical cancer. For metastatic cervical cancers (stage IVB), and recurrent cervical cancer patients who cannot be effectively treated using radiation or surgery, chemotherapy is the primary treatment (74). However, in spite of the compliance to these therapeutic regimes, up to 70 % of the cancer patients experience pelvic recurrence of tumor, distant metastases, or both along with an increasing chemoresistance that ultimately culminates into death of the patient (75).

6. EMERGING THERAPEUTICS

The major challenge for emerging immune- or antiviral-based therapies for HPV-associated conditions is to safely provide a clear advantage over any existing treatment; particularly for late precancer lesions like CIN2/3 where treatment efficacy is 90–95%. Keeping in view spontaneous regression of CIN2/3 lesions non-surgical methods are always preferred over unnecessary surgical interventions that lead to patient anxiety and risk for premature delivery in case of a reproductively active woman. Apart from effective utilization of clinically available treatment modalities, there has been an extensive research for the treatment and management of advanced cervical cancer and HPV infection using immunomodulators, viral-derived proteins or small molecule inhibitors which can be broadly and interchangeably classified as immunotherapeutics, anti-viral therapeutics, or anti-cancer therapeutics without discrete boundaries.

6.1. Immune checkpoint inhibitors and repurposed anti-cancer drugs

Use of T cell immune checkpoint inhibitors is emerging as a promising approach in clinical research for treatment of cervical cancer. These inhibitors target molecules that regulate T cell immune responses. Several different checkpoints inhibitors particularly the

monoclonal antibodies that target specific antigens on tumors, are currently in different developmental phases like Ipilimumab [(anti-CTLA-4 antibody) NCT01711515], Pembrolizumab [(PD-1 antibody) NCT02628067], and Durvalumab [programmed death ligand-1 (PD-L1) NCT01975831]. Bevacizumab that targets vascular endothelial growth factor (VEGF) prevents angiogenesis, is USFDA approved for the treatment of late-stage or recurrent cervical cancer. Other similar inhibitors in clinical trials are HuMax®-TF-ADC. This is drug-conjugated antibody targeting tissue factor-specific cells, and is being evaluated in clinical trials in patients with cervical or other advanced cancers (NCT02001623, NCT02552121). Another drug-conjugate antibody, IMMU-132 that target human trophoblast cell-surface antigen TROP-2 ectopically expressed in a variety of cancers, is being evaluated in a phase I/II clinical trial in patients with advanced cancer, including cervical cancer (76). Several T cell based-immunotherapy approaches are being examined in clinical trials for cervical cancers and have shown promise (76). Again, most of these therapeutic approaches are not specific to HPV infection.

In parallel to these attempts, HPV-specific treatment strategies are being developed that can be broadly classified into therapeutic vaccines that boost anti-viral immune mechanisms or curtail viral addition of host cell by targeting its oncogenes by gene silencing using RNA-based strategies or more radical newly emerging genome editing approaches, as well as by use of herbal derivatives that come as pure compounds, extracts, polyherbals or as formulations. Research carried out in areas has been summarized in the following sections.

6.2. Therapeutic HPV vaccines

As specific anti-HPV approaches, therapeutic vaccines are being examined to treat HPV-associated pre-cancers and cancer lesions where HPV infection is already established. As opposed to prophylactic vaccines that induce neutralizing antibodies against L1 capsid proteins, therapeutic vaccines are targeted against viral E6 and E7 oncoproteins that are expressed throughout the life cycle of the virus, as other viral proteins like L1/L2 are either not expressed or deleted/inactivated due to viral integration (E1 and E2) in transformed tumor cells. Therapeutic vaccines can be broadly classified as (a) live bacterial or viral vector based (77, 78); (b) peptide or protein based (79, 80); nucleic acid based (81, 82); and whole cell based (83, 84) [reviewed in (85–87)]. Although the list of therapeutic vaccines is rapidly increasing as more than 20 trials are in ongoing phase I/II stage whereas some interventions have already completed phase II/III. HPV therapeutic vaccines recently evaluated in stage II or III of clinical evaluation have been listed in Table 1. Among these

Table 1. Leading anti-HPV therapeutic vaccines recently evaluated in Phase III or Phase II of clinical evaluation

Vaccine Name, Target Antigens, Organization (Vaccine Type)	Disease Model	Immune Response	Outcome on Lesion	Outcome on HPV Infection	Side Effects Reported	Ref./ Clinical Trial ID
Phase III						
MVA E2 HPV-16 E2 Instituto Mexicano del Seguro Social (Viral Vector-based)	Patients with HPV-induced AGIN (n=1176 female and n=180 male)	Antibody against tumor cells and HPV-specific CD4+ and CD8+ T cells in patients who successfully eliminated previous HPV 16 infections	90% lesion clearance in female treated patient and 100% lesion clearance in male	HPV DNA was not detected after treatment in 83% of total patients treated.	No major side effects observed	(88, 89)
Phase II						
VGX 3100 HPV16/18 E6/E7 Inovio Pharma (DNA- based)	HPV16/18 positive CIN2/3 Patients (n=167)	Immune responses in peripheral blood (both CD8+ T cell and antibody) and Vaccinations enhance T cell and humoral response.	49.5% vaccinated patient demonstrated regression compared to 30.6. % in placebo group	ND	Injection site reaction, fatigue, headache, myalgia, nausea, arthralgia, erythema	(81)/ (92) NCT01304524
HPV16-SLP HPV16 E6/E7 ISA Pharma (Protein-based)	Patients with low-grade abnormalities of the cervix (n=50)	HPV16-specific T-cell response as well as to establish long-term immunologic memory	ND	97 % of vaccinated patients generated HPV 16-specific CMI	Flu-like symptom, injection site reaction	(80)
	Patients with HPV16+ advanced or recurrent gynecological carcinoma (n=20)	Duration of survival correlated with magnitude of T cell response with production of IFN γ , TNF α , IL-5 and/or IL-10 and 9 patients with HPV16-specific immune response	No regression of tumors was observed among the 12 evaluable patients	9 out of 16 tested patients induced HPV16-specific proliferative responses	Injection site reaction, fever, chills, fatigue, nausea, flu-like symptom	(79)
	Patients with HPV16+ HSIL (n=9)	HPV-specific T cell response	All vaccinated patients showed strong response after vaccination, change in patterns of immune infiltrate	No HPV clearance at the time of LEEP excision	Inflammation and pain at injection site, headache, diarrhea, fatigue, rash, dizziness, nausea, chills, myalgia, fever, urticaria	(94)
	Patients with HPV16+ VIN3 (n=20)	Circulating HPV-16 specific T cells	15 patients had objective Clinical response at 12 months. 9 complete responses and 6 partial response	83 % had CMI against HPV-16	Local swelling, redness, increased skin temp, pain at vaccination site, fever, flu like symptoms, chills, and tiredness	(95) / (93) NCT02128126
GLBL101c HPV16 E7 GENOLAC BL Corp (Bacterial Vector- based)	HPV16+ CIN3 patients (n=17)	Significant increase in E7-CMI in cervical vaginal tract	9 patients experienced disease regression to CIN2, and 5 further regressed to LSIL	ND	No major side effects observed	(77)/ UMIN000012229/ NCT02195089
TA-CIN HPV16 E6E7L2 Xenova Res Ltd. (Fusion protein-based)	VIN2/3 patients (n=19)	Cell subsets and lymphocyte proliferation for HPV systemic immune responses	63 % lesion response 1 year after vaccination. Significant increase. Significant CMI observed in lesion responders	HPV16 clearance and 79% (15 out of 19)	Local reaction associated With Imiquimod.	(96)

Anti-HPV therapeutics: Translatable leads

TA-CIN + TA-HPV HPV16/18 L2/E6/E7 Celtic Pharma (Fusion protein-based)	HPV16+ high-grade AGIN Patients (n=29)	17 patients showed TA-CIN induced T cell responses. 11 generated HPV-16/18 E6 and/or E7 specific T cells. 14 with IgG response to HPV-16 E7.	ND	ND	ND	(97)
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Abbreviations: CIN – Cervical Intraepithelial Neoplasia; ICC – Invasive carcinoma of the cervix; ND – Not determined; VIN – Vaginal Intraepithelial Neoplasia

MVA E2 targeted against HPV16E2 has successfully completed Phase III trial with 90% to 100% remission of anogenital intraepithelial neoplasia (AGIN) lesions in women and men respectively, and HPV clearance was observed in 83% cases in total patients (88). Earlier this vaccine showed 94% efficacy in treating CIN1/2/3 lesions (89), whereas in high grade lesions (CIN2/3) its complete remission was in 56% and partial remission in 32% (90). The vaccine was also useful in treatment of flat condylomas in men leading to eradication of lesion and HPV in 93% of vaccine administered cases (91). Interestingly, MVA E2 is a vaccinia virus Ankara (MVA) containing the bovine papillomavirus E2 protein, but not the expected oncoproteins E6, E7 or E5 of human counterpart of the vaccine strain. As mentioned earlier, expression of E6 and E7 oncoproteins is controlled by E2 that exerts a negative regulatory function on early promoter that transcribes these oncogenes. Therefore, reintroduction of E2 suppressed the expression of E6 and E7 transcripts in HPV-infected cells, and subsequently reduced the transforming ability of the infected, malignant HPV-associated tumor cells (87).

Other vaccines that completed phase II clinical trial are DNA-based VXG3100 (81, 92), protein-based HPV16 E6/E7 Synthetic Long Peptide (79, 80, 93–95), bacterial vector-based GLBL1010c (77), fusion protein-based TA-CIN (96) and TA-CIN+TA-HPV vaccines (97). Most of these vaccines except orally-administered GLBL101c, have local mild to moderate side effects (Table 1). Further, HPV16 SLP vaccine tested in HPV16 positive advanced recurrent gynaecological carcinoma, 9 out of 16 tested patients induced HPV16-specific proliferative response (79). No regression of tumors was observed among the 12 evaluable patients. On the other hand, whole cell based dendritic cell vaccines which hold a lot of promise (98), have proven to be weakly immunogenic in two of the completed phase I studies (83, 84). Apart from these, T-cell based vaccines, involving increase of tumor reactivity of tumor infiltrating lymphocytes using defined antigens in different phases of clinical development pipeline [listed in (87)], and leaving one study (99) the results of these on-going clinical trials are yet to become public. Most of these vaccines showed an efficacy associated with the induction of a strong HPV-specific CD4+ and CD8+ T cell activity. Careful

analysis of patients with unresponsive lesions showed reduced systemic vaccine responses but an increased numbers of lesion associated immune suppressive T regulatory cells (Tregs) (93, 100). The failure to cure some premalignant lesions and the cancers appears to result from an unfavorable balance in effector T cells and Tregs. The increased understanding of the role of immune regulation in prevention of effective anti-tumor responses, particularly optimal use of adjuvants in vaccines leading to adequate infiltration of lesion by immune effector cells, may be an adjunct strategy to improve the clinical outcome of the immunotherapeutic regimens in future.

6.3. HPV genome targeting strategies

With emergence of genetic engineering and evolution of gene manipulation technologies, strategies involving nucleic acid (NA) – based targeting of specific HPV gene(s) are now increasingly gaining recognition in both experimental and clinical research. Early NA-based anti-viral agents included antisense oligonucleotides (AS-ODN), ribozymes (Rz) & DNAzymes (Dz), short interfering RNA (siRNA), and short hairpin RNA (shRNA). These therapeutic sequences were designed to target specifically the E6 and E7 regions of HR-HPV genome or their mRNAs, leading to specific, strong and effective down regulation of viral oncogene expression. Recent evolution of gene specific editing technologies utilizing zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the clustered regularly interspaced short palindromic repeat-associated nuclease (CRISPR/Cas9) RNA-guided endonuclease has resulted in further expansion of specific, robust and precise targeting of HPV genome of infected cells and could provide a long lasting solution particularly in advanced stages where the HPV genome is already integrated in host cell. Clinical implementation of these therapies, however, demands a parallel progress in suitable vectors and intracellular targeted delivery of the NA-based therapeutics to the infected cells. The most lucrative aspect that derives this segment of research is high probability to generate revenues as the reagents created are custom-made and may lead to generation of intellectual property. Specific HPV genome targeting approaches employed in different studies are summarized in Table 2. Each of

Table 2. Targeting of HPV gene expression and genome using molecular approaches and gene manipulation technology

Target, Location and Constructs	Study Type	Delivery Method, Combination	Biological Response	Ref.
RNA targeting approaches using Anti-Sense Oligonucleotides (AS-ODNs)				
HPV16 & HPV18 E6 and E7 (start site of E6 and E7)	<i>In vitro</i> , 1483, C4–1, CaSki, SiHa and HeLa	Sterile water/culture medium	<ul style="list-style-type: none"> Growth inhibition of cervical and oral cancer cells harboring HPV18 or HPV16 but had little effect on cells lacking HPV Inhibited formation of colonies in soft agar 	(102, 103)
HPV16 & HPV18 E6 and E7	<i>In vivo</i> in nude mice	Transfection (DOTAP)	<ul style="list-style-type: none"> Substantially reduced tumor formation in nude mice. AE7 inhibited E7 synthesis and AE6 ODN suppressed the expression of E6 as well as E7 	(103)
HPV16 E6 and E7 (full gene in antisense orientation)	<i>In vitro</i> SiHa; <i>In vivo</i> in nude mice	Adenovirus-mediated Ad5CMV-HPV 16 AS	<ul style="list-style-type: none"> Growth of infected cells suppressed, decreased cell count E6 and E7 protein expression suppressed, and p53 and Rb protein expression increased, cells underwent apoptosis <i>in vitro</i> and <i>in vivo</i> Tumorigenicity was completely inhibited in mice injected infected SiHa cells 	(109, 110)
HPV16 E6 and E7 (plasmid pU6E7AS with U6/antisense chimeric genes)	<i>In vitro</i> Hu 293, C33A Mu C3 cells <i>In vivo</i> C3 tumors	Cationic liposome-mediated transfection	<ul style="list-style-type: none"> Down-regulated E7 gene expression Intra-tumor injection of plasmid resulted in significant growth inhibition of HPV16-positive C3 tumors Potential of plasmids antitumor immunity by co-delivery of IL-12 gene in mouse tumor 	(106, 107)
HPV-16 E6 (nt 410–445)	<i>In vitro</i> CaSki, QGU, SiHa; <i>In vivo</i> in nude mice	Sterile water <i>in vitro</i> ; osmotic pumps <i>in vivo</i>	<ul style="list-style-type: none"> Efficient growth inhibition of monolayer and agar-growth HPV-16-containing tumor cell lines in a dose-dependent manner Inhibited tumor growth in transplanted nude mice Inhibition of anchorage independent growth 	(104, 105)
HPV16 E6/E7 (fast-hybridizing RNA segments)	<i>In vitro</i> , SiHa	Transfection (DOTAP)	<ul style="list-style-type: none"> Growth inhibition of cultured cells Biological effects of antisense ODNs were specific for the antisense sequence 	(268)
HPV16 E7 (FL E7AS cDNA)	<i>In vitro</i> CaSki; <i>in vivo</i> CaSki in nude mice	pBabe-puro or pWZL-Hygro retrovirus vectors	<ul style="list-style-type: none"> Reduced HPV16 E7 protein expression and cell proliferation, cell cycle arrest, up-regulated RB, and down-regulated E2F-1 and bcl-2 proteins Retarded the tumorigenicity of CaSki cells <i>in vivo</i> 	(111)
HPV 16 E6 (plasmid containing E6AS)	<i>In vitro</i> CaSki, SiHa	Transfection	<ul style="list-style-type: none"> inhibited E6 splicing, rapidly upregulated p53 and a p53-responsive protein, GADD45 and induced apoptosis and related changes 	(108)
HPV 16 E7 (first 100nt)	<i>In-vitro</i> CaSki	Non-neuro-invasive HSV1	<ul style="list-style-type: none"> HPV-16 E7 protein expression was most efficiently downregulated with R5226 recombinant virus. 	(112)
HPV 16 E6 & E7 (AS-RNA)	<i>In vitro</i> SiHa	Transfection	<ul style="list-style-type: none"> Repressed HPV16 E6 and E7 transcripts Led to apoptosis and replicative senescence of tumor cells. Increase of both p53 expression and hypo-phosphorylated p105Rb 	(269)
RNA targeting approaches using catalytic nucleotides: Ribozymes (Rz) and DNAzymes (Dz)				
HPV16 E6 and E7 (nt 110 and 558; nt 240 and 597) Hammerheaded Rz	<i>In-vitro</i> Cell free conditions;	synthetic and transcribed Rz from RNA-trimming plasmid pRG523 or CWR7:SVN	<ul style="list-style-type: none"> Efficient cleavage of HPV-16 E6/E7 transcripts Conditions like ionic strength, Mg⁺⁺ concentration and temperature affected catalysis 	(115, 116)
HPV18 E6 and E7 (nt 123, 309, and 671) Hammerheaded Rz	<i>In-vitro</i> Cell free conditions HeLa; E. coli with HPV and ribozyme transgene	Transfection/transformation, use of helper phage (T7/M13)	<ul style="list-style-type: none"> Each ribozyme hybridized to its target site in cell free conditions with maximal hybridization within 1 hour. HPV RNA from the HeLa was cleaved effectively by each ribozyme. Simultaneous expression in Escherichia coli, each ribozyme produced a significant reduction in the intracellular concentration of HPV RNA. The ribozyme directed to nt 309 was the most effective. Cell growth reduction, increased serum dependency, and reduced foci formation in soft agar 	(117, 118)
HPV16-E6/E7 (R434) HairPin Rz, Triplex HairPin	<i>In vitro</i> Cell free conditions and normal human keratinocytes	Transfection	<ul style="list-style-type: none"> Efficiently inhibited E6 <i>in vitro</i> translation Reduced the growth rate and prevented immortalization of E6/E7 transformed normal human keratinocytes 	(119, 121, 122)

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HPV6b/11E1 (nt 1198)	<i>In vitro</i> Cell free conditions	-	<ul style="list-style-type: none"> • Possessed the perfect specific catalytic cleavage activity in cell free assays. 	(270)
HPV 16 E6/E7 Rz170	<i>in vitro</i> CaSki and <i>in vivo</i> CaSki in nude mice	Transfection	<ul style="list-style-type: none"> • Rz170 Stably Expressed in CaSki Cells reduced expression of HPV16 E6E7 mRNA • Rz170 Inhibits CaSki-R Cell Growth <i>In vitro</i> and <i>In vivo</i> • Rz170 Increases Apoptosis in Cells by Changing Expression of Viral and Cellular Proteins. 	(120)
HPV16 E6/E7 (nt 410–445) Dz1023–434	Cell free conditions and <i>in vitro</i> CaSki	Transfection	<ul style="list-style-type: none"> • Showed efficient cleavage within HPV16 E6/E7 mRNA even in low (Mg²⁺) • Decreased intracellular E6/E7 mRNA levels in HPV16-positive cells • Decreased proliferation and induced cell death 	(123, 124)
RNA targeting approaches using Short-interfering RNAs (siRNA) and short hairpin RNA (shRNA)				
HPV16 E6 and E7 (nt 224–242)	<i>In vitro</i> CaSki, SiHa	Transfection by liposomes	<ul style="list-style-type: none"> • E6 silencing induced accumulation of cellular p53 protein, transactivation of the cell cycle control p21 gene and reduced cell growth. • E7 silencing induced hypo-phosphorylation of retinoblastoma protein and apoptotic cell death. 	(133)
HPV16/18 E6 and E7 (nt 385–403)	<i>In vitro</i> HeLa, SiHa	pSUPER vector born with calcium phosphate co-precipitation and synthetic by transfection by oligofectamine	<ul style="list-style-type: none"> • Both vector-borne and synthetic siRNAs against HPV E6 restored dormant tumor suppressor pathways (p53, pRB) • Resulted in massive apoptotic cell death selectively in HPV-positive tumor cells. 	(134)
HPV18 E7 (142 to 160 nt of E7 coding sequence)	<i>In vitro</i> HeLa,	Transfection by oligofectamine	<ul style="list-style-type: none"> • Inhibited cellular DNA synthesis • Induced morphological and biochemical changes characteristic of cellular senescence 	(135)
HPV16 E6 (nt 385–403)	<i>In vitro</i> SiHa; <i>in vivo</i> in nude mice	Transfection by oligofectamine	<ul style="list-style-type: none"> • Decreased the levels of E6 and E7 mRNA • Nuclear accumulation of p53 with p21 (CIP1/WAF1) induction and hypo-phosphorylation of retinoblastoma protein • Suppressed monolayer and anchorage-independent growth • Tumors in NOD/SCID mice that were significantly smaller than in those treated with control siRNA. 	(136)
HPV18 E6 (18E6–165, 18E6–340, and 18E6–385) and HPV18 E7 (18E7–604 and 18E7–694)	<i>In vitro</i> HeLa,	Transfection by oligofectamine; adjunct to cisplatin, carboplatin, doxorubicin, etoposide, topotecan, gemcitabine, mitomycin, mitoxantrone, oxaliplatin, and paclitaxel	<ul style="list-style-type: none"> • Treatment with E6 siRNA alone moderately inhibited HeLa cell proliferation but did not induce detectable apoptosis. • The combined cytotoxic effect of E6 siRNA and chemotherapy ranged from sub-additive to synergistic, depending on the drug • Sensitized cells to doxorubicin and gemcitabine but counteracted the cytotoxicity of cisplatin and etoposide. 	(137)
HPV16 E6 (siRNA123–131, 455–473) and HPV18 E6 (shRNA nt 128–145)	<i>In vitro</i> CaSki, HeLa	Transfection by oligofectamine; adjunct to cisplatin	<ul style="list-style-type: none"> • Reduction in cellular viability concurrent with the induction of cellular senescence by siRNA • Marked increase in the levels of p53 after co-treatment with E6 siRNA and cisplatin and decrease chemosensitivity • Addition of a lentivirus-delivered shRNA, the IC50 was reduced almost 4-fold to 2.4 μM in HeLa cells 	(138)
HPV16 E6	<i>In vitro</i> CaSki;	Transfection by liposome <i>in vitro</i> ; siRNA injected i.p. or s.c.	<ul style="list-style-type: none"> • Apoptosis rate of CaSki cells at days 1, 2, 5, and 9 after siRNA transfection were 7.7 %, 11.8 %, 37.4 %, and 12.6 %, respectively. • The HPV16 E6 mRNA level reduced by 77%, 83%, 59%, and 41% • E6 siRNA administered mice showed inhibited tumor growth, suppressed E6 protein level, and necrotic tumors with apoptosis 	(139)
HPV18 E6 (18E6–165, 18E6–340)	<i>in vivo</i> in nude mice SKG-II xenografts	Intratumoral injected with Atelocollagen (AteloGene) <i>in vivo</i>	<ul style="list-style-type: none"> • Cell growth inhibition with decreased expression of E6 and E7 mRNA • Treatment of xenografts suppressed tumor growth <i>in vivo</i> 	(140)
HPV16 E6 (A-D) shRNA	<i>In vitro</i>	shRNA expression vector	<ul style="list-style-type: none"> • Resulted in stable and specific silencing of E6mRNA • Accumulation of p53, p21, and hypo-phosphorylated pRb protein • Cell proliferation, colony formation ability, tumorigenicity, and <i>in vitro</i> cell invasive capability were suppressed 	(141)

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HPV18 E6 and E7 shRNA (18E6–1 targets at the common sites of all mRNA classes, whereas 18E6–2 targets only class I mRNA)	<i>In vitro</i> HeLa; <i>in vivo</i> HeLa xenografts in nude mice; HeLa lung metastasis model	Infection with polybrene; Lentiviral vector plasmid pLentiLox3.7	<ul style="list-style-type: none"> Low dose shRNA reduced cell growth and the induction of senescence, high-dose infection resulted in specific cell death via apoptosis <i>in vitro</i> HeLa cells infected with a low dose of LV-shRNA significantly reduced the tumor weight, whereas high dose resulted in a complete loss of tumor growth. Systemic delivery into mice with established HeLa cell lung metastases reduced the number of tumor nodules 	(142)
HPV 16 E6	<i>In vitro</i> CaSki, SiHa	Transfection by liposome; Co-delivery with monoclonal Antibody	<ul style="list-style-type: none"> Cell growth was almost totally suppressed by co-delivery. Antibodies and siRNA act synergistically when co-delivered. 	(143)
HPV 18 E6 and E7	<i>In vitro</i> HeLa and C4-I	Transfection	<ul style="list-style-type: none"> Re-expressed p53 protein and a moderate decrease in phosphor pRb Decreased colony formation and increased apoptotic cells 	(144)
HPV16 E7 (nt 564–584) shRNA	<i>In vitro</i> CaSki, SiHa	shRNA In U6 promoter-driven vector pSIREN-DNR-DsRed-Expression Vector	<ul style="list-style-type: none"> Targeting HPV16-E7 region degraded E6, truncated E6 (E6*) and E7 mRNAs and simultaneously knockdown both E6 and E7 expression Accumulation of cellular p53 and p21 and induced hypo-phosphorylation of Rb protein Induction of apoptosis in treated cells 	(145)
HPV16 E6 & E7 (nt 497–519, 573–595; 752–774)	<i>In vitro</i> CaSki, SiHa; <i>in vivo</i> in nude mice	Transfection <i>in vitro</i> ; Complex with Atelocollagen (AteloGene) <i>in vivo</i>	<ul style="list-style-type: none"> Growth suppression and cellular senescence accompanied by accumulation of p53 and p21WAF1/CIP1 Antitumor activity confirmed by retarded tumor growth of HPV16+ cells in intratumorally- injected NOD/SCID mice 	(146)
HPV16 E6 & E7	<i>In vitro</i> CaSki; <i>in vivo</i> xenografts in nude mice	Transfection <i>in vitro</i> ; Intratumoral injection <i>in vivo</i>	<ul style="list-style-type: none"> 67% and 71% reduction in E6/E7 mRNA Extended treatment till 35 days with siRNA completely or nearly eradicated tumors in 70% of the animals 	(147)
HPV16 E6 & E7 (nt155–173, 224–242, and 662–680)	<i>In vitro</i> CaSki, SiHa; <i>in vivo</i> SiHa xenografts in nude mice	Transfection <i>in vitro</i> ; adjunct to paclitaxel, carboplatin, irinotecan, leptomycin B or doxorubicin, cisplatin	<ul style="list-style-type: none"> Reduced Cellular proliferation; the effect being greater in SiHa than CaSki Carboplatin, irinotecan, leptomycin B or doxorubicin led to additive toxicity, whereas, with cisplatin, led to sub-additive toxicity Paclitaxel resulted in synergistic toxicity with intronic siRNA (E6) Tumors reduced using paclitaxel siRNA E6 & E7mix Synergistic relationship between p53 restoration and paclitaxel 	(148)
HPV16 E6 and E7 shRNA	<i>In vitro</i> CaSki and TC1 cells; <i>in vivo</i> TC-1	Transfection by Lipofectamine, Transduction by HPV pseudovirions, Lentivirus delivery	<ul style="list-style-type: none"> Degradation of E6 and E7 mRNAs by E6 and E7 shRNA delivered by pseudovirions E6 silencing resulted in p53 accumulation and reduced cell viability. Targeting E7 resulted in high apoptosis Lentivirus and HPV virus-like particle vectors coding for an E7 shRNA sequence both resulted in dramatic inhibition of tumor growth. 	(149)
HPV16 E6/E7 (nt 249–267, 164–183, 161–178, 666–682, 629–645, 628–649) HPV18 E6/E7 (119–125, 666–682, 674–690)	<i>In vitro</i> CaSki, HeLa; <i>in vivo</i> SiHa xenografts in nude mice	TOPO2 plasmid vectors, Transfection <i>in vitro</i> ; Intratumoral injection <i>in vivo</i>	<ul style="list-style-type: none"> Reduced cell growth and colony formation with increased apoptosis Intratumoral injection of the siRNAs reduced tumor growth in nude mice 	(150)
HPV16 E6 & E7 (nt 497–519, 573–595; 752–774) double stranded DNA-RNA Chimera (dsRDC)	<i>In vitro</i> SiHa, E6E7-immortalized human keratinocytes,	Transfection;	<ul style="list-style-type: none"> dsRDC modification reduced nonspecific cytotoxicity of 497 and 752 and their off-target effects. dsRDC modification mildly affected their silencing activity dsRDC-497 induced E6E7-specific growth suppression 	(151)
HPV16 E6 & E7	<i>In vitro</i> SiHa,	Transfection <i>in vitro</i> ; adjunct to TRAIL	<ul style="list-style-type: none"> Significant upregulation of death receptors DR4 and DR5 but did not result in an enhanced sensitivity to TRAIL Occurrence of siRNA induced senescence may prevent TRAIL-mediated death 	(152)

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HPV16 E6/E7	<i>in vivo</i> xenografts in nude mice TC-1	Systemic delivery (i.v.) of DOTAP/DOPE liposomes; Adjunct to Cisplatin	<ul style="list-style-type: none"> 50% reduction in tumor size with siRNA alone comparable to cisplatin alone No improvement in combination with cisplatin 	(153)
HPV18 E6 or E6/E7, HPV16 E6/E7 (497)	<i>In vitro</i> HeLa, SiHa, CaSki; <i>in vivo</i> xenografts in nude mice	Transfection cationic liposome; Adjunct to Cisplatin (CDDP)	<ul style="list-style-type: none"> Combination induced superior apoptosis and cellular senescence <i>in vitro</i> E6/E7-specific siRNA potentiated the antitumor efficacy of CDDP via induction of apoptosis, senescence and anti-angiogenesis 	(154)
HPV16 E6/E7	<i>In vitro</i> HeLa, SiHa	Oligofectamine transfection; adjunct to cisplatin, irradiation, rhTRAIL, or anti-Fas antibody	<ul style="list-style-type: none"> E6 suppression conferred susceptibility to cisplatin-induced apoptosis but not to irradiation-, rhTRAIL-, or anti-Fas-induced apoptosis Combining cisplatin with rhTRAIL or anti-Fas induced higher apoptosis Cisplatin elevated p53 levels, enhanced caspase-3 activation, and reduced p21 levels in E6-suppressed cells. 	(155)
HPV 18 E6/E7	<i>In vitro</i> Uveal melanoma cell line, VUP	Oligofectamine transfection	<ul style="list-style-type: none"> Growth inhibition and cell cycle block by activation of the p53 and Rb pathways 	(156)
HPV16 E6 (nt109–127,288–306) and E7 (nt101–119) shRNA	<i>In vitro</i> SiHa; <i>in vivo</i> SiHa xenografts in nude mice	Transfection lipofectamine; pSilencer 1.0.-U6 plasmid; co-express with p53 wild type	<ul style="list-style-type: none"> Simultaneous expression of pSi-E6-P53 caused a robust suppression of tumor growth Tumor growth inhibition occurred due to tumor cell death <i>in vivo</i> pSi-E6-P53 resulted in anti-angiogenesis in grafted tumor tissues 	(157)
HPV16 E6 and E7 (pools of 3–5 different target-specific non-overlapping siRNA of 19–25 nt)	<i>In vitro</i> SiHa	RNAiMax transfection	<ul style="list-style-type: none"> E6 specific siRNA resulted in increased Let-7a but loss of miR-21 and a correspondingly reduced pSTAT3/STAT3 and elevated the level of cellular PTEN. E6 siRNA resulted in reduced GLI1 transcripts and stem cell signaling Showed differential involvement of HPV E6 in EMT manifestation 	(50, 53, 158)
HPV16 E6 (pools of 3–5 different target-specific non-overlapping siRNA of 19–25 nt)	<i>In vitro</i> Stem cells isolated from SiHa	RNAiMax transfection	<ul style="list-style-type: none"> siRNA silencing of HPV16 E6 abolished sphere formation, downregulated AP-1-STAT3 signaling, and induced re-differentiation 	(51)
HPV Genome Targeting approaches using Zinc Finger Nuclease (ZFN)				
HPV18 E2 (AZP-SNase)	<i>In vitro</i> HeLa	Fusion with cell permeable peptides	<ul style="list-style-type: none"> Artificial zinc finger nuclease cleaved the target replication origin site on HPV DNA in cell. This is confirmed by Ligation mediated PCR. Potential drawback- the SNase cleaves RNA and single-stranded DNA (ssDNA) as well as double-stranded DNA (dsDNA) 	(172)
HPV18 E2 (AZP-scFokI)	<i>In vitro</i> HeLa	Transfection lipofectamine	<ul style="list-style-type: none"> Artificial zinc finger nuclease cleaved the target replication origin site on HPV DNA in cell with no toxicity 	(173)
HPV16 and 18 E7	<i>In vitro</i> SiHa, HeLa <i>In vivo</i> nude mice SiHa, HeLa xenografts	ZFNs plasmids with TurboFect <i>in vivo</i> Transfection Reagent	<ul style="list-style-type: none"> ZFN16-E7-S2 and ZFN18-E7-S2 disrupted HPV E7 oncogenes in HPV16/18–positive cervical cancer cells ZFNs inhibited type-specific cell growth, and induced apoptosis ZFN repressed xenograft formation 	(174)
HPV Genome Targeting approaches based on TALEN				
HPV16 E6, E7	<i>in vitro</i> HeLa, SiHa, <i>in vivo</i> K14-HPV16 female mouse model	TALEN plasmid in X-tremeGENE HP DNA Transfection Reagent for <i>in vitro</i> ; complexed with Turbo-Fect <i>In vivo</i> Transfection Reagent for <i>in vivo</i>	<ul style="list-style-type: none"> TALEN-mediated genome editing of HPV oncogenes E6/E7 efficiently disrupted E6 and E7 oncogenes, successfully triggered specific apoptosis and growth inhibition and reduced tumorigenicity of HPV-infected cells. TALEN led to restoration of p53 and RB1 Direct cervical application of HPV16-E7–targeted TALENs effectively mutated the E7 oncogene, reduced viral DNA load, and restored RB1 function and downstream E2F1 and cyclin dependent kinase 2 (CDK2) and reversed the malignant phenotype. 	(176)

HPV Genome Targeting approaches based on CRISPR-Cas9				
HPV16-E7 (sgRNA - DSB at 564, 583, 616, 688)	<i>in vitro</i> SiHa, CaSki	Transfection with X-tremeGENE HP DNA Transfection Reagent	<ul style="list-style-type: none"> Guided CRISPR/Cas system disrupted HPV16-E7 DNA at specific sites, inducing apoptosis and growth inhibition in HPV positive cells Disruption of E7 DNA directly led to downregulation of E7 and upregulation of pRb 	(179)
HPV16 & 18 E6 & E7 (sgRNA)	<i>In vitro</i> SiHa, HeLa	Calcium phosphate transfection method	<ul style="list-style-type: none"> Induce cleavage of the HPV genome, resulting in the introduction of inactivating deletion and insertion mutations into the E6 or E7 gene Induction of p53 or Rb, led to cell cycle arrest and eventual cell death Both HPV-16- and HPV-18-transformed cells were found to be responsive to targeted HPV genome-specific DNA cleavage 	(180)
HPV16 E6 (sgRNA - DSB at 268, 404, 507)	<i>in vitro</i> SiHa, CaSki	Transfection with X-tremeGENE HP DNA Transfection Reagent	<ul style="list-style-type: none"> Cleaved at specific sites, leading to apoptosis and growth inhibition of HPV16-positive cells Downregulated E6 protein and restored p53 protein 	(181)
HPV6/11 E7	<i>In vitro</i> E7-transformed keratinocytes	Transfection with Lipofectamine 2000	<ul style="list-style-type: none"> Both HPV6/11 E7 genes can be inactivated by the single CRISPR-Cas9 system Silencing of E7 led to inhibition of cell proliferation and induction of apoptosis in E7-transformed keratinocytes 	(182)
HPV18 sgRNA5170, sgRNA36	<i>In vitro</i> HeLa	sgRNA/Cas9 dual expression vector pSpCas9	<ul style="list-style-type: none"> Knocking out of the integrated HPV fragment in HeLa cell line decreased expression of MYC located ~500 kb downstream of the integration site Integrated HPV fragment influence MYC expression via long distance chromatin interaction 	(183)

DOTAP: N- (1- (2,3-Di-oleoyloxy) propyl)-N,N,N-trimethyl ammonium methylsulfate; Rz: ribozyme; sgRNA - single-guide RNA; TC-1 cells - murine C57B/6 lung epithelial cells transformed with HPV16 E6/7 and ras oncogenes; TRAIL – tumor necrosis factor-related apoptosis-inducing ligand.

these endeavors has consumed nearly three decades of scientific effort, but still it is very much a work-in-progress.

6.3.1. Silencing HPV RNA by antisense oligonucleotides (AS-ODN)

Use of antisense approach started with testing of native antisense oligonucleotides (AS-ODN) (101). Because of the intracellular stability issues, AS-ODNs were subsequently modified in their phosphate group to phosphorothioate, which made them resistant to intracellular exonucleases (102–105). These AS-ODNs worked efficiently and could inhibit HPV genes by translational silencing, target degradation with the help of endogenous (nuclear) RNaseH, or potentially by exon skipping. Unique feature of these molecules was that they did not require any specific delivery system. However, these early approaches required large quantities of ODNs. Alternatively, different expression vector constructs were employed using plasmids (106–108), adeno- (109, 110) and retroviral vectors (111, 112). This considerably improved duration of antisense DNA/RNA mediated silencing and helped in avoiding repeat applications in *in vivo* settings. These approaches were effective in *in vitro* and to some extent in *in vivo* experimental setups but essentially required additional delivery methods that are generally not suitable to implement in patients. Further apart, the off target effects were also frequently

noted while working with these reagents. Incidentally, use of AS-ODNs which is otherwise very promising molecular therapy remains untouched for clinical evaluation due to parallel advancements in other gene silencing technologies (i.e. siRNA and shRNAs). A wide array of strategic questions pertaining to the medicinal chemistry of oligonucleotides, pharmacokinetics, toxicology, as well as the molecular pharmacology of AS-OSN, remain unaddressed.

6.3.2. Silencing HPV RNA by catalytic oligonucleotides

Another equally promising experimental approach to target HPV transcripts has been using specifically engineered oligonucleotides with trans-acting catalytic properties (113). Commonly referred to as ribozymes and DNAzymes based on their ribose or deoxyribose sugar backbone, both lead to site-specific cleavage of target mRNAs but considerably differ in origin of their catalytic core, mechanism of action and stability within the system (114). Both, engineered ribozymes and recently the DNAzymes have been examined against HPV (Table 2). Among these, about 60 nucleotide long hammerhead ribozymes were designed first and showed activity in cell free system or *in vitro* cultured cells lines (115–118). Due to size and other experimental variables, research shifted to hairpin ribozymes that were significantly smaller in size (30 nucleotides) (119). A number of ribozyme

targets were discovered in viral transcripts, among them R434 and R309 were the most effective target sites reported in HPV16 and HPV18, respectively in some studies (117–119). Ribozyme targeting HPV16 E6E7 transcripts (Rz170) suppressed cell growth and sensitized cervical cancer cells to chemotherapy and radiotherapy (120). To counteract size related problems due to cloning in plasmid vectors, the construct was modified to triplex ribozyme expression system that released processed ribozymes as a single transcript by a self-processing mechanism (121, 122). However, these modifications did not increase ribozyme mediated inhibition more than 30% (122), and thus this approach required further improvement to make ribozymes a therapeutic moiety. Further, the catalytic activity the ribozymes found to dramatically drop within the cellular environment due to Mg^{++} availability, nuclease action protein binding and with reduced probability of co-localization with the target. Because of deleterious off-target effects in intracellular milieu of an otherwise specific and proven catalytic molecule against HPV in biochemical assays, the pursuit to make this class of molecules as potential therapeutics faded considerably. To overcome problems encountered with ribozymes, alternative molecules particularly the DNAzymes were explored (123, 124). DNAzymes combined the efficiency of ribozymes and intracellular stability and simplicity of AS-ODNs (125). Out of the two commonly used DNAzymes, a widely used and well-characterized 10–23 motif has been engineered to destroy HPV oncogenes (123, 124). DNAzyme Dz1023–434 showed efficient cleavage against HPV-16 E6/E7 mRNA within bona fide antisense window at nt 410–445 even in low (Mg^{++}) condition cell free systems. The stability of these molecules was further improved by locked nucleic acid (LNA)-modification with specific cleavage of HPV transcripts in cell cultures. Despite these improvements, current significance of ribozymes does not look very promising in the light of the emergence of new powerful, simple and easy to use anti-mRNA strategies, particularly the RNA interference (RNAi).

6.3.3. Silencing HPV RNA by RNA interference

This technology chiefly relies on perfect (or nearly perfect) matching of specific 2–8nt RNA sequences known as ‘seed’ regions that are naturally present on 22nt (± 4 nt) long guide RNA in RISC complex ubiquitously present in all eukaryotic cells. These small interfering RNAs (siRNA) recognize and guide degradation of the target mRNA, or lead to its translational arrest depending upon the degree of complementarity (126). By exogenously supplementing synthetic siRNA, the naturally existing RNAi machinery can be manipulated to silence a desired mRNA with precision (127). The efficiency of the process is several orders of magnitude more than antisense or ribozyme treatment (128), though its utility is also restricted by its

dependence on acceptable delivery methods that are rapidly evolving (129). siRNA technology has evolved extensively over last decade leading to its evaluation in several clinical trials including some cancers, but targeting HPV or cervical cancer by siRNA is yet to be attempted in clinical setting and it is expected anytime. There have been periodic reviews that evaluated the therapeutic potential of siRNA in treating HPV-associated malignant disease as well as evaluation of the progress in its delivery methods (130–132), and only salient finding of the studies are being discussed.

Efforts to develop siRNA-based anti-HPV therapeutics have been entirely focused around E6 and E7 transcripts of the high risk HPV16 and HPV18 (50, 51, 53, 133–158) (Table 2). A superior silencing was observed if the E6 specific monoclonal antibodies were co-delivered with siRNA in intra-cytoplasmic compartment using liposomes (143). These studies have exhaustively utilized HPV16- (SiHa, CaSki) and HPV18-positive cells (HeLa, C4-I) or murine TC-1 cells (lung epithelial cells transformed with HPV16 E6/7 and ras oncogenes) both *in vitro* and *in vivo* as xenografts in nude mice. Despite nucleotide modifications that improved the intracellular stability and off target effects (159) targeting of E6 or E7 by siRNA has been a transient phenomenon and limits its use *in vivo*. The challenge was mitigated by utilizing shRNA approach (138, 141, 142, 145, 149, 157) or by making double-stranded RNA–DNA chimera (dsRNADNA) (151). Though theoretical off target effects of siRNA seed sequences are high which raised concern, experimental studies show lack of any correlation between predicted and actual off-target effects of siRNAs targeting the HPV16 E7 oncogene (160). Further, expression of shRNA in cells is stable and induces robust silencing for a long period ranging beyond 4 months *in vitro* (141). However, this strategy typically requires delivery through viral (142, 149) or bacterial expression vectors (134, 141, 145, 150, 157) which can pose safety concerns (161).

Targeting E6 or E7 by siRNA and snRNA invariably resulted in concomitant reemergence of p53 and phospho-pRB pools that were accompanied by a variable extent of a spectrum of anti-cancer effects characterized by (i) reduced cell growth in monolayers and in anchorage-independent conditions, (ii) arrested cell cycle and DNA synthesis, (iii) reduced colony forming ability, (iv) induction of senescence and apoptosis (v) reduced invasion and expression of epithelial mesenchymal transition markers, and (vi) loss of stemness and associated signaling in cultured cells (Table 2). Similarly, when these siRNA were administered *in vivo* in xenografted HPV-positive cells that form tumors in nude mice, tumorigenicity of treated cells declined and a reduced tumor formation was invariably noticed along with reduced angiogenesis and metastatic potential but to a variable

extent. These variations were largely dependent on dose of siRNA, delivery methods, vectors, route of administration, or the transcript region against which the siRNA were raised.

Parallel to the development in siRNA technology, studies were carried out to integrate this promising RNAi approach with conventional and targeted anti-cancer therapy against cervical cancer (137, 148, 152–155). Combination studies performed in *in vitro* cultures showed a variable wide-ranging response from synergistic to additive to sub-additive within different studies. However, most of the combinations failed in animal models. As siRNAs are polyanions that do not readily cross the cell membrane *in vivo* coupled with immune-hostile tumor microenvironment and physiological barriers of the circulatory system, susceptibility to ribonuclease degradation, rapid renal excretion and nonspecific uptake by the reticulo-endothelial system, delivery of siRNAs remains a major obstacle to their clinical or even *in vivo* use (162). These bottlenecks are currently guiding research to develop safe, stable, and efficient nano-engineered targeted delivery systems some of which are currently under clinical testing (132).

6.3.4. HPV Genome Targeting approaches

Targeting DNA through triplex forming oligonucleotides (TFO) was the initial and oldest attempt for transcriptional interference of HPV (163). Subsequently, several natural triplex forming sites were reported in HPV genome (164). Though DNA triplexes exhibited remarkable sequence specificity (165) and stability (166), the approach did not affect HPV replication or transcription appreciably. Among highly popular and easy to use RNA interference approaches, there has been emergence of a revolutionary research in specific targeted DNA manipulation techniques that led to HPV genome editing. These include ZFNs, TALENs, and the CRISPR/Cas9RNA-guided endonuclease system. Each of these technologies specifically recognize and locate a target sequence with homologous binding specific protein domains or the guide RNA (gRNA) and catalyze a DNA double stranded break by attached restriction enzyme and reviewed extensively in recent past (167). Because of the precision of programmable nucleases these technologies are proposed as a powerful antiviral therapy for persistent viral infections (168, 169). Proof-of-concept studies have been performed to treat multiple disorders, including *in vivo* experiments in mammals and even early phase human trials (NCT01455389, NCT00595088, and NCT01118052).

ZFN were the first among engineer customized zinc-finger proteins created by fusing DNA-binding zinc finger proteins to the FokI DNA-cleavage domain (170) and are protected by patent to Sangamo Biosciences

that resulted prohibitive costs in negotiating multiple use (171). Two custom-designed ZFNs catalyze cleavage in both strands upon binding to DNA in dimeric form that activates FokI nuclease. Double-strand breaks (DSB) created by ZFNs are repaired primarily by non-homologous end joining pathway in the absence of any other similar (donor) fragment. In case these enzymes are co-delivered with a donor template that directs gene replacement, the two DSBs are repaired by the homology-directed repair pathway. Early ZFN targeted against HPV were engineered for HPV18 E2 region which prevented replication of the virus in the cell (172, 173). Subsequently, engineered FokI-based constructs were developed against HPV16 and HPV18 E7 (174). These ZFNs disrupted HPV E7 oncogenes in both HPV16/18–positive cervical cancer cells, led to cell growth inhibition and induced apoptosis of corresponding HPV16- and HPV18-positive cervical cancer cell lines. ZFNs also repressed xenograft formation *in vivo*. Further work in this direction is less due to patent restrictions associated with this valuable technology.

Like ZFN, TALENs contain two domains out of which one depend upon repeat variable diresidues (RVD) that are arranged in 33–35 amino acid tandem repeat arrays which determine the specificity of the protein domain and can be engineered to recognize specific target sequence, and the other is FokI nuclease. Compared to ZFNs, TALENs possess several advantages including lower cytotoxicity, greater design flexibility, and simple engineering (175). Recently, TALEN-mediated targeting of HPV oncogenes was found to ameliorate HPV-related cervical malignancy (176). TALENs disrupted E6 and E7 oncogenes, successfully triggered specific apoptosis and growth inhibition and reduced tumorigenicity of HPV-infected cells *in vitro*, and mutated the E7 oncogene, reduced viral DNA load, and restored RB1 function and downstream E2F1 and CDK2, and reversed the malignant phenotype in K14 HPV16 transgenic female mice when administered *in vivo* directly at cervix. TALENs have their own limitations. The numbers of exogenous sequences that can be targeted are limited by the use of a thymidine at 4th position (177). Further, methylcytosine is not cleaved by the conventional TALENs (178). Methylcytosine is indistinguishable from thymidine in major groove which reduces target specificity. Further, TALEN repeats are difficult to amplify in PCR due to repeats and are susceptible to recombination.

The CRISPR/Cas system consists of two components: guide gRNA and nucleases (Cas). This originally constitutes the immune system of some bacteria and used for defending against foreign nucleic acids. The most commonly used type is CRISPR/Cas9 including a conjugation of the tracrRNA (trans-activating crRNA)—crRNA (CRISPR RNA) to a

single guide RNA and Cas9 (175). Unlike ZFNs and TALENs that involve engineering protein domains, this system utilizes a simple guide RNA that directs DSB in DNA and the specificity can be changed by simply altering the guide RNA in the complex. Recently the CRISPR/Cas9 system has emerged as a promising candidate against HPV (179–183) (Table 2). Both high risk HPV16 and HPV18 as well as low risk HPV6 and HPV11 have been targeted with reasonable efficiency. These genome editing experiments also resulted in loss of correspondingly targeted genes and associated downstream events. Interestingly, recent study showed targeting of a large HPV genome fragment can result in restoration of gene functions of integration-targeted cellular genes thus facilitating reversal of the HPV integration event (183).

Even though HPV genome editing is a promising approach, properties, like negative charge, large size, low membrane penetration, low endosomal escape, and weak serum tolerance, genome editing by these nucleases prevent successful implementation of the technology unless an effective *in vivo* delivery system is developed for these programmable nucleases. Potential delivery strategies under clinical testing and commonly used vectors available and suitable for each class of nucleases are discussed elsewhere (175) and are equally applicable in the context of HPV genome editing. Further, the obstacles being faced in testing of gene editing-based antiviral therapeutics in animal models and transition towards human application have discussed in detail elsewhere (169). Even if these operational problems could be resolved, a major impediment to the widespread application of gene editing and gene manipulation based antivirals will be the potential cost to the populations at risk and will push development of alternate low cost therapeutics for treatment of target population which usually belongs to low socio-economic strata and cannot afford this sophisticated technology.

6.4. Emerging anti-HPV pharmaceuticals from nature

As per the survey conducted by National Cancer Institute (USA), of 175 approved anti-tumor drugs 131 are non-synthetic and are either natural products, their mimics or directly derived from them (184, 185). Even the standard anti-cancer therapeutics like vincristine, vinblastine, paclitaxel and camptothecin as well as podophyllotoxin, which are the established treatments for genital warts, are of plant origin (186). Therefore, in addition to developing immunotherapeutics, another major area of active research for developing treatments against cervical cancer is to explore potential pharmaceutical agents from natural resources especially plants. Ever since the cultures of HeLa cells were developed in

1952 (187, 188), the most practiced approach that prevailed prior to the knowledge of viral etiology of cervical cancer, was to test potential drugs or phytochemicals on this established cervical cancer cell line. Many natural products have frequently been tested and were found to possess potentially useful anti-cancer activities that have been summarized in Table 3. However, majority of these investigational entities displayed generic anti-cancer effects and the investigations remained restricted to testing them in the cell lines. These leads should be screened further to explore if they have any HPV-specific effects and their potential utility in clinical settings if feasible need to be established. Nevertheless, a sizable effort has been directed towards specific pharmacological targeting of HPV-mediated events during cervical carcinogenesis that can be broadly grouped together as (a) prevention of virus entry into the host cell; (b) to eliminate virally-infected cervical cells or to reduce their viral load in precancer stage; (c) to prevent virus-induced tumorigenic transformation; (d) abrogate carcinogenic progression by reducing expression of high risk oncoproteins E6/E7 or rendering them non-functional, (e) targeting host factors that are essentially required for expression of viral oncogenes or elimination of oxidative stress leading to apoptotic cell death of cervical cancer cells; (f) boosting immune surveillance or inhibiting co-infections that work as cofactors, and/or (f) to improve the efficacy of standard chemo-radiation therapies.

Natural products demonstrated to have anti-HPV activities and can emerge as potential treatments for cervical cancer are listed in Table 4. These natural products can be broadly classified as (a) phytochemicals which are mostly available as cataloged purified and characterized chemical entities, (b) isolated active compounds from crude or fractionated extracts, or (c) the extracts themselves that are used as such for treatment of cervical cells *in vitro* and are poorly defined and hard to reproduce, or (d) polyherbals having different constituent herbal derivatives with pharmacologically approved excipients that contain a combination of pure compounds or extracts and are the most complex formulations among all. Individual anti-viral and anticancer activities and drug interaction between components of these formulations are poorly defined. Keeping in view the space constraints, only those natural products that showed specific anti-HPV effects have been discussed in detail (Table 4).

Among phytochemicals, curcumin (diferulylmethane) derived from *Curcuma longa* Linn. displayed strong anti-HPV activity by targeting the expression of viral oncogenes (44, 45, 189). Curcumin is a known potent anti-inflammatory and anti-cancer small molecule inhibitor with proven pharmacological safety (190). The mechanistic studies revealed the inhibitory effect on oncogenes transcription was

Table 3. Molecular targeting of cervical cancer using anti-carcinogenic properties of natural/herbal formulation, pure compound, plant extract and polyphenols

Investigational Drug / Entity (Type)	Activity	Type of Study/ Experimental Model/ Study Design	Cell type/Model/ Clinical	Outcome/ Key Observations/ Molecular Targets/ Mechanism of Action	Ref.
Pure Compound/isolated chemicals/phytochemicals against cervical cancer					
Indole-3-carbinol (I3C)	Anti-estrogenic activities	<i>In vitro</i>	CaSki	<ul style="list-style-type: none"> Estradiol increased expression of HPV oncogenes I3C has anti-estrogenic activities which prevent cancer in cervical cells 	(271)
Curcumin	Anti-Cancer	Phase I	N=25, 13 men and 12 women, with a median age of 60 year, 2 patients with recently resected bladder cancer, 7 patients with oral leukoplakia, 6 patients with intestinal meta Plasia of the stomach, 4 patients with CIN and 6 patients with Bowen's disease.	<ul style="list-style-type: none"> Curcumin showed a chemo-preventive effect Safe dose of curcumin is upto 8 mg by day 	(193)
	Anti-inflammatory	<i>In vitro</i>	HeLa, <i>in situ</i> and (n=78) cervical tissue sample	<ul style="list-style-type: none"> Statistically significant difference in the expression of cox-2 Low doses of curcumin to cervical cancer cells induces significant changes in tumor-related proteins 	(192)
	Antiproliferation and Induction of apoptosis	<i>In vitro</i>	HeLa SiHa CaSki	<ul style="list-style-type: none"> Downregulation of Bcl-2, Bcl-XL, COX-2 Upregulation of Bax, AIF, release of cytochrome c 	(191)
	Antiproliferation and Induction of apoptosis	<i>In vitro</i>	HeLa, SiHa, CaSki, and C33A	<ul style="list-style-type: none"> Overcome the proliferative effect of estradiol by causing decrease in level of PCNA, Cyclin D1, and viral oncoprotein E7 	(272)
Emodin (1,3,8-trihydroxy-6-methylanthraquinone)	Antiproliferative effect	<i>In vitro</i>	Bu 25TK cells	<ul style="list-style-type: none"> Inhibited DNA synthesis Induced apoptosis as demonstrated by increased nuclear condensation, annexin binding and DNA fragmentation 	(273)
Daidzein	Antiproliferative properties	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Expression of human telomerase catalytic subunit mRNA decreased Affected cell growth, cell cycle and telomerase activity <i>in vitro</i> 	(274)
Epigallocatechin gallate (EGCG)	Antiproliferative properties	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Inhibition of proliferation with suppression of pKi-67 and telomerase activity and induction of apoptosis 	(275)
	Induction of apoptosis	<i>In vitro</i>	OMC-4, TMCC-1	<ul style="list-style-type: none"> Inhibition of HPV16 transcription by inhibition SP1-mediated transcription Stabilization of p53 	(276)
	Antiproliferation	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Combination of EGCG with RA induced apoptosis Inhibited telomerase activity 	(277)
	Antioxidant, Anti-proliferative, Inhibitory effects on angiogenesis and Tumor cell invasion	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Inhibited invasion Migration of HeLa cells and modulated the expression of related genes (MMP-9 and TIMP-1) 	(278)
	Antiproliferation, Induction of apoptosis And Anti-invasion	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Down-regulation of genes involved in the stimulation of proliferation, adhesion and motility as well as invasion processes, Up-regulation of several genes known to have antagonist effects. Reduced proliferation rates, adhesion and spreading ability as well as invasiveness 	(279)

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Chrysin	Antiproliferation	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Effectively inhibit growth by down-regulated expression of PCNA Induce apoptosis 	(248)
Apigenin (4',5,7,-trihydroxyflavone)	Anti-invasive effect	<i>In vitro/ in vivo</i>	HeLa Cx43, HeLa and chick embryo heart fragments	<ul style="list-style-type: none"> Tumor cell invasiveness Inhibition of tumor cell motility 	(280)
Apigeninn	Anti-cervical	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Decreased in the protein expression of Bcl-2 protein; induced p53 expression Down regulation of Bcl-2 expression 	(281)
	Induction of apoptosis	<i>In vitro</i>	CaSki, HeLa and C33A	<ul style="list-style-type: none"> Inhibits cervical cancer cell growth through the induction of apoptosis 	(282)
Genistein	Anti-cervical	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Activated pAKT (Thr 308) was inhibited enhancement of the radiation effect that may be partially mediated by G (2)M arrest Mcl-1 and activation of the AKT gene Migration-inhibition in a time-dependent manner by modulating the expression of MMP-9 and TIMP-1 	(228)
Rhodoxanthin	Antiproliferation	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Reduction of the mitochondria transmembrane potential Increase of the intracellular Ca²⁺ concentration and accumulation of cells in the S phase 	(283)
ATA	Antiproliferation	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Downregulation of Bcl-2 expression Upregulation of Bax expression Activation of the caspase-3 pathway 	(284)
Oridonin	Antiproliferation	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Downregulation of the protein kinase B (Akt) activation Expression of FOX transcription factor and GSK3 	(285)
HMBBJ (p-hydroxy-methoxybenzo-bijuglone)	Antiproliferation	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Downregulation of Bcl-2 expression Upregulate Bax expression and increase of sub-G1 group 	(286)
Trichosanthin	Antiproliferation	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Decrease of the amount of actin mRNA Down-regulation of β-tubulin mRNAs 	(287)
Soyasaponins	Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Induction of apoptosis through the mitochondrial pathway 	(288)
Solanum nigrum Phytoglycoprotein	Anti-cervical		HeLa	<ul style="list-style-type: none"> Reduces the binding activities of NF-κB and AP-1 and declines the level of Nitric Oxide (NO) production 	(289)
Abrus agglutinin (peptide fraction)	Antiproliferation and Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Induced ROS generation Decreased Bcl-2/Bax ratio to elicit mitochondrial permeability transition Activate caspase-3, finally leading to DNA fragmentation and cell apoptosis 	(290)
Anonaine	Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Upregulation of Bax and p53 proteins expression Increase of intracellular NO, ROS, glutathione depletion Disruptive mitochondrial transmembrane potential 	(291)
Mannose-Binding Lectin	Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Typical caspase-dependent apoptotic mechanism 	(292)
Nucleases CMN1 and CMN2	Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Induction of expression of proteins responsible for apoptosis execution 	(293)
Parviflorene F	Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Enhancement of mRNA and protein expression of TRAIL-R2 Activation of caspase-8,-9 and -3 	(294)
Clitocine	Antiproliferation and Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Downregulation of Bcl-2 and upregulation of Bax Release of cytochrome c and activation of caspase-3 	(295)

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Cyanidin 3-glucoside	Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> • Not investigated 	(296)
Kaempferol-7-O- -D-glucoside	Antiproliferation	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> • Induction of G2/M phase growth arrest • Decrease of cyclin B1 and CDK1 • Inhibition of NF-κB nuclear translocation • Upregulation of Bax and downregulation of Bcl-2 	(297)
Tan IIA	Antiproliferation and Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> • Induction of mitotic arrest and apoptosis through the JNK-mediated mitochondrial pathway 	(298)
Zerumbone	Cyto-selective toxicity Antiproliferation	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> • Increase of the level of caspase-3, induction of G2/M phase cell cycle arrest • Inhibition of the level of IL-6 in cancer cells 	(299)
Coumarin A/AA	Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> • Activation of an apoptosis-like cell death program • Release of the pro-apoptotic protein AIF, without disturbance of cell cycle 	(300)
Camptothecin	Induction of apoptosis and Antiproliferation	<i>In vitro</i>	SiHa	<ul style="list-style-type: none"> • loss of mitochondrial membrane potential • Decreased Bcl-2 levels, cytochrome c release, caspase-3 activation • Formation of reactive oxygen species and depletion of GS 	(301)
Isoliquiritigenin	Antiproliferation	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> • Induction of G2/M phase cell cycle arrest, increase of p21 expression in a p53-dependent manner and decrease of cdc2, cdc25C and cyclin B expression • Regulation of the Bcl-2 family protein expression • Phosphorylates Chk2 and subsequently increases the accumulation of inactive cdc25C and cdc2 	(302)
Resveratrol and Roscovitine	Antiproliferation	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> • Arrested cell population faster into G2/M phase • Induction of apoptosis 	(303)
Oroxylin A	Induction of apoptosis	<i>In vitro/ in vivo</i>	HeLa	<ul style="list-style-type: none"> • Decrease of Bcl-2 protein expression and degradation of • PARP 	(304)
Nebrodeolysin	Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> • Exhibited remarkable hemolytic activity toward rabbit erythrocytes, caused efflux of potassium ions from erythrocytes • Induced apoptosis in HeLa cells 	(305)
HY253	Induction of apoptosis Antiproliferation	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> • Downregulation of anti-apoptotic Bcl-2 expression • Increase of cell population in the sub-G1 phase • Activation of caspase-3, 7, 8, and 9 and degradation of PARP protein 	(306)
Isoliquiritigenin (4,20,40-trihydroxychalcone)	Antiproliferation	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> • Induction of cell cycle arrest in both the G2 and M phases via inhibition of topoisomerase II activity • Regulation of DSB-mediated ATM/Chk2 signaling pathway in HeLa cells 	(307)
23-Hydroxyursolic acid	Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> • Decrease of Bcl-XL and Bcl-2 expression and NF-κB p65 • protein level 	(308)
CRA (Corosolic acid)	Antiproliferation Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> • Induction of apoptosis via activation of the caspase-dependent mitochondrial pathway 	(309)
Astragalus (mongholicus lectin)	Antiproliferation Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> • Induction of S-phase arrest • Upregulation of p21 and p27 and reduction of active complex cyclin E/CDK2 kinase formation 	(310)

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DPPT (Deoxypodophyllotoxin)	Antiproliferation Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> • Inhibition of tubulin polymerization and regulation of cyclin A and cyclin B1 expression • Activation of caspases-3 and -7 	(311)
Astilbotriterpenic acid	Antiproliferation Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> • Induction of caspase activation, release of ROS • Downregulation of Bcl-2 and upregulation of Bax 	(312)
APS-1d (a novel polysaccharide)	Antiproliferation Induction of apoptosis	<i>In vitro/in vivo</i>	HeLa	<ul style="list-style-type: none"> • Regulation of Bcl-2 family protein expression, decrease of the mitochondrial membrane potential • Increase of the cytosolic cytochrome c level and caspase-9, -3 and PARP activities 	(313)
18-Hydroxyferruginol (Hinokiol, kayadiol)	Antiproliferation Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> • Increase of Bax/Bcl-2 ratio • Depolarization of mitochondrial membrane potential 	(314)
Ginger	Induction of apoptosis and Antiproliferation	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> • Inhibition of microtubule structure and functions • Increase of cell population in sub-G0/G1 phase 	(315)
Eupafolin	Antiproliferation Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> • Induction of apoptosis through the caspase-dependent pathway 	(316)
Garcinia paucinervis	Induction of apoptosis	<i>In vitro</i>	HeLa-C3	<ul style="list-style-type: none"> • Activated caspase-3, exhibited the strongest inhibitory effect against HeLa cell growth among 	(317)
Pinellia pedatisecta Schott (PE)	Induction of apoptosis Antiproliferation	<i>In vitro</i>	CaSki, HeLa, HBL-100	<ul style="list-style-type: none"> • Down-regulate E6 gene expression at both mRNA and protein levels • Activate p53 and induce apoptosis • Inhibit cell proliferation 	(204)
Lycopodine	Antiproliferation Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> • Induction of chromatin condensation and internucleosomal DNA • Fragmentation • Enhancement of cell population in sub-G1 region • Increase of ROS generation and mitochondrial membrane potential depolarization, release of cytochrome c and activation of caspase-3 	(261)
Cannabidiol	Anti-invasion	<i>In vitro</i>	HeLa, C33A	<ul style="list-style-type: none"> • The decrease of invasion by upregulation of TIMP-1 Knockdown of cannabidiol-induced TIMP-1 expression by siRNA led to a reversal of the cannabidiol-elicited decrease in tumor cell invasiveness 	(318)
Saikosaponin	Induction of apoptosis	<i>In vitro</i>	HeLa, SiHa	<ul style="list-style-type: none"> • Induction of cellular ROS accumulation mediates synergistic cytotoxicity in saikosaponins and cisplatin co-treated cancer cells 	(218)
3 alpha,23- isopropylidenedioxyolean- 12-en-27-oic acid	Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> • Release of cytochrome c, activation of caspase-9 • Increase of ER stress, GPR78 and GADD153 activation, Ca²⁺ release and activation of calpain 	(319)
Gallic acid	Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> • Induction of cell death via apoptosis • Necrosis was accompanied by ROS increase and GSH depletion 	(320)
Quercetin	Anti-cervical	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> • Induction of G2/M phase cell cycle arrest and mitochondrial apoptosis • Inhibition of anti-apoptotic AKT and Bcl-2 expression 	(321)
Epigallocatechin gallate and Polyphenols E	Anti-cervical	<i>In vitro</i>	Me180 and HeLa	<ul style="list-style-type: none"> • Inhibited immortalized cervical epithelial and cancer cell growth • HPV-E7 protein expression was decreased 	(322)

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Paclitaxel and Curcumin	Induced apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Down-regulation of paclitaxel-induced NF-κB activation upon Akt inactivation 	(216)
Heterofucan	Induction of apoptosis Antiproliferation	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Release of mitochondrial AIF into cytosol Decrease of Bcl-2 expression and increase of Bax expression 	(323)
1H- (1,2,4)oxadiazolo (4,3-a)quinoxalin-1-one (ODQ)	Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Inhibit the polymerization of purified tubulin Inhibits microtubule assembly by direct oxidation of tubulin Induces cell cycle arrest at the G2/M phase, and triggers apoptosis 	(324)
Xanthone V1 and 2-acetyl-1,4-naphthoquinone	Antiproliferation and Induction of apoptosis	<i>In vitro</i>	HeLa, CaSki	<ul style="list-style-type: none"> Cell cycle distribution Apoptosis induction, caspase 3/7 activation and the anti-angiogenic properties 	(325)
Liquiritigenin	Antiproliferation Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Upregulation of p53, release of cytochrome c and elevation of caspase-9 and -3 activities 	(326)
Ganoderic acid Mf and ganoderic acid S	Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Induction of apoptosis through the mitochondria- dependent pathway 	(327)
Withaferin A	Induction of apoptosis, Antiproliferation and target oncogenes	<i>In vitro/ in vivo</i>	C33A, HeLa, CaSki and SiHa Nude mice	<ul style="list-style-type: none"> Repress HPV E6 and E7 oncogenes Downregulates HPV oncoproteins and restores p53 levels Inhibits STAT3 signaling proteins and modulates p53-dependent apoptotic markers 	(328)
Thymoquinone	Induction of apoptosis	<i>In vitro</i>	SiHa	<ul style="list-style-type: none"> Elevation of p53 and downregulation of the Bcl-2 protein 	(329)
Enhydrin, Uvedalin and Sonchifolin	Antiproliferation and Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Increase of the caspase-3/7 activation Inhibition of the NF- κB binding protein activation 	(330)
Isoflavone	Antiproliferation Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Induction of apoptosis through the mitochondrial pathway 	(331)
Decursin and decursinol angelate	Antiproliferation Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Activation of caspases, cleavage of PARP, increase of TRAIL and TRAIL receptors expression Regulation of the Bcl-2, Bcl-XL, survivin, cIAP-1, -2 and XIAP expression 	(222)
Zerumbone	Cytoselective toxicity and Antiproliferation	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Increase of the level of caspase-3 Induction of G2/M phase cell cycle arrest and inhibition of the level of IL-6 in cancer cells 	(332)
Oblongifolin C	Induction of apoptosis Antiproliferation	<i>In vitro/ In vivo</i>	HeLa	<ul style="list-style-type: none"> Inhibit the growth of xenografted tumors Induction of Bax translocation Cytochrome c release, mitochondrial fission and swelling Reduction of mitochondrial membrane potential 	(333)
(+)-40-Decanoyl-cis-khellactone and (+)-30-decanoyl-cis-khellactone	Antiproliferation and Induction of apoptosis	<i>In vitro</i>	HeLa, SiHa and C-33A	<ul style="list-style-type: none"> (+)-40-Decanoyl-cis-khellactone elicited apoptosis via both extrinsic and intrinsic pathways (+)-30-decanoyl-cis- khellactone induced apoptosis only by the intrinsic pathway Both of them acted as an anticancer supplement by inducing cell cycle arrest in the S/G2 phase and caspase- dependent apoptosis 	(334)
3,4',5 tri-hydroxystilbene (resveratrol)	Anti-invasion	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Inhibits both NF-κB and AP-1 mediated MMP-9 expression Leading to suppression of migration and invasion of human metastatic cervical cancer cells 	(335)

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AgNPs	Induction of apoptosis and Increase of life span	<i>In vitro</i> <i>in vivo</i>	HeLa	<ul style="list-style-type: none"> Disruption of the mitochondrial respiratory chain Increase of ROS production and interruption of ATP synthesis and causing DNA damage 	(243)
Stigmasterol, Beccamarin	Antiproliferation	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Not investigated 	(336)
Luteolin	Induction of apoptosis and Inhibition of tumor growth	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Luteolin sensitized HeLa cells to TRAIL-induced apoptosis by both extrinsic and intrinsic apoptotic pathways 	(337)
Fisetin	Antiproliferation ,Induction of apoptosis and Significantly reduced tumor growth	<i>In vitro</i> <i>in vivo</i>	HeLa and Tumor-xenografted nude mice model	<ul style="list-style-type: none"> Significantly reduced tumor growth Activation of the phosphorylation ERK1/2 PD98059 Activation of caspase-8/-3 pathway 	(338)
Silymarin	Anti-Cancer	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Apoptosis by no-mitochondrial pathway mediated through p38/ JNK MAPKs 	(339)
	Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Downregulation of Bcl-2 and caspase-3 protein expression 	(340)
Silibinin	Antiproliferation and Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Induction of G2 arrest and the decrease in CDKs involved in both G1 and G2 progression Elicitation of apoptosis via both the mitochondrial and death receptor-mediated pathways 	(341)
Chelidonine	Anti-cervical and Anti-cancer	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> p38-p53 and PI3K/AKT signaling pathways 	(342)
Kaempferitrin	Induction of apoptosis	<i>In vitro</i> <i>in vivo</i>	HeLa	<ul style="list-style-type: none"> Decrease of body weight Increase of the survival Induction of cell cycle arrest in G1 phase Apoptosis via the caspase-dependent intrinsic pathway 	(343)
Hesperetin	Induction of apoptosis	<i>In vitro</i>	SiHa	<ul style="list-style-type: none"> Inhibition of proliferation and induced the G2/M phase An attenuation of mitochondrial membrane potential with increased expression of caspase-3, caspase-8, caspase-9, p53, Bax, and Fas death receptor Adaptor protein Fas-associated death domain-containing protein (FADD) 	(344)
Carandinol	Cytotoxicity	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Not investigated 	(345)
Resveratrol	Induction of apoptosis	<i>In vitro</i>	C33A, HeLa, CaSki and SiHa	<ul style="list-style-type: none"> Decreased expression of p53 Decreased expression of p65 (an NF-κB subunit) 	(346)
Celastrol	Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Depolarization of mitochondrial membrane potential Increased expression of caspase-3 	(347)
Green synthesis of AgNPs	Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Induction of cell death through a ROS-mediated apoptotic process 	(348)
30-Hydroxy-11a-methoxy-18b-olean-12-en-3-one	Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Served as a mediator of the ROS scavenging system 	(349)
Naringin (NRG)	Antiproliferation and Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Induction of apoptosis through both death-receptor and mitochondrial pathways 	(350)
	Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Reduced activation of NF-κB/ COX-2-caspase-1 	(351)

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Hesperetin	Anti-cervical	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Attenuation of mitochondrial membrane potential with increased expression of caspase-3, caspase-8, caspase-9, p53, Bax Fas death receptor and its adaptor protein Fas-associated death domain-containing protein (FADD) induced apoptosis was confirmed by TUNEL and Annexin V-Cy3 	(344)
Biosynthesis of AgNPs	Cytotoxicity	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Not investigated 	(244)
MCPT	Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Induction of apoptosis via extrinsic and intrinsic apoptotic pathways 	(352)
Amygdalin	Anti-cervical and Anti-cancer	<i>In vitro/ in vivo</i>	HeLa	<ul style="list-style-type: none"> Induction apoptosis via increase in caspase-3 activity Anti-apoptotic protein Bcl-2 was downregulated Pro-apoptotic Bax protein upregulation 	(353)
Condurango	Anti-cervical and Anti-cancer	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Apoptosis via ROS-dependent p53 signaling pathway 	(354)
	Anti-cervical and Anti-cancer	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Epigenetic Modification through acetylation/deacetylation of histones 	(355)
Conium maculatu	Anti-cervical and Anti-cancer	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Induce apoptosis 	(262)
Dihydroartemisinin (DHA)	Induces apoptosis	<i>In vitro and In vivo</i>	HeLa, CaSki and Nude mice	<ul style="list-style-type: none"> Upregulation of RKIP mRNA and protein Significant downregulation of bcl-2 mRNA and protein 	(356)
Extracts against cervical cancer					
Pterocarpus santalinus	Induction of apoptosis and Antiproliferation	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Release of cytochrome c activation of caspases-9 and -3 and degradation of PARP 	(357)
Corallina pilulifera	Induction of apoptosis Antiproliferation	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Induction of apoptosis through the mitochondria-dependent pathway Downregulation of DNA topoisomerase IIa gene expression 	(358)
Cremanthodium humile (C. humile)	Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Collapse of mitochondrial membrane potential, release of cytochrome c Activation of caspase-3/7 and -9 ROS-mediated mitochondrial dysfunction pathway 	(359)
Saffron	Induction of apoptosis and Antiproliferation	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Induction of a sub-G1 peak and ROS production 	(360)
Wheat sprout	Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Induction of all proteasome activities gradual inhibition 	(361)
wheat bud (peptides)	Antiproliferation	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Induction of DNA damage and G2 arrest Inactivation of the CDK1-cyclin B1 complex and increase of active chk1 kinase expression 	(362)
Duchesnea indica (Andr.)	Induction of apoptosis	<i>in vitro and in vivo</i>	HeLa, C33A and U14	<ul style="list-style-type: none"> Induction apoptosis via increase in caspase-3 activity Antiapoptotic protein Bcl-2 was downregulated Proapoptotic Bax protein upregulation 	(363)
Cassia tora	Induction of apoptosis Antiproliferation	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Reduction of DNA content and caspase -3 activity 	(364)
Nigella sativa	Immune-modulatory, Antiproliferation and Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Regulation of the expression of pro- and anti-apoptotic 	(365)

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Cordyceps Pruinosa	Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Downregulation of Bcl-2 expression Activation of caspases and degradation of PARP protein 	(366)
	Induction of apoptosis and Antiproliferation	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Decrease of Bcl-2 protein, increase of Bax protein, release of cytochrome c and AIF Activation of caspases -3 and -9 	(366)
Cinnamomum Cassia	Induction of apoptosis	<i>In vitro</i>	SiHa	<ul style="list-style-type: none"> Increase of intracellular calcium signaling as well as loss of mitochondrial membrane potential Downregulation of MMP-2 expression to reduce migration potential 	(367)
Scutellaria lindbergi	Antiproliferation	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Induction of the sub-G1 peak 	(368)
Black Raspberry	Antiproliferation	<i>In vitro</i>	HeLa, SiHa, C-33A	<ul style="list-style-type: none"> Not investigated 	(369)
Croton lechleri Mull. Arg. (Euphorbiaceae)	Cytotoxicity and Decrease of body weight	<i>In vitro/ in vivo</i>	HeLa	<ul style="list-style-type: none"> Not investigated 	(370)
Mango Peel	Induction of apoptosis and Antiproliferation	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Downregulation of anti-apoptotic Bcl-2 expression Increase of cell population in the sub-G1 phase Activation of caspase-3, 7, 8, and 9 and degradation of PARP protein 	(371)
Ficus hirta Vahl. (Wuzhimaotao)	Antiproliferation	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Inhibition of cell viability, induction of morphology changes Increase of sub-G1 phase 	(372)
Artemisia afra Jacq.	Antiproliferation and Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Induction of caspase activation Cell cycle arrest in the G2/M phase 	(373)
Derivatives of phytochemicals					
Methylenedioxy Lignan	Antiproliferation	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Inhibiting telomerase and activation of c-myc Caspases leading to apoptosis 	(254)
Diethyl chysin-7-yl phosphate (CPE: C19H19O7P) and Tetraethyl bis-phosphoric ester of chrysin (CP: C23H28O10P2) CR derivative	Antiproliferation	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Induction of tumor cell apoptosis Downregulation of PCNA expression (tumor malignancy) 	(248)
2 -Nitroflavone	Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Induction of apoptosis through both death receptor Mitochondria-dependent pathways 	(249)
PG (3,4,5-trihydroxybenzoic acid propyl ester)	Antiproliferation and Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Inhibition of HeLa cells growth via caspase-dependent apoptosis as well as cell cycle arrest 	(250)
3- and 10-bromofascaplysins	Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Induction of caspase-8, -9, -3-dependent apoptosis 	(251)
Butylated Hydroxyanisole	Antiproliferation and Induction of apoptosis	<i>In vitro</i>	HeLa	<ul style="list-style-type: none"> Induction of caspase- dependent apoptosis Increase of GSH depletion and O2-level 	(252)
Diethyl 5,7,40 -trihydroxy flavanone N-phenyl hydrazone (N101-2)	Antiproliferation	<i>In vitro</i>	SiHa, CaSki	<ul style="list-style-type: none"> Induction of apoptosis by arresting cell cycle at sub-G1 phase Activation of mitochondria-emanated intrinsic and Fas-mediated extrinsic signaling pathways Inhibition of the PI3K/AKT pathway 	(253)

Table 4: Molecular targeting of HPV infection using natural/herbal products, phytochemicals and their analogs, pure compound, plant extract and their formulations

Investigational Drug / Entity	Activity	Type of Study/ Experimental Model/ Study Design	Cell type/Model/ Clinical	Outcome/ Key Observations/ Molecular Targets/ Mechanism of Action	Ref.
Phytochemicals/Natural products					
Curcumin (in vaginal gelatin capsules)	Elimination of HPV DNA/ HPV-infected cells	Phase I/II, randomized double blind	HPV positive women without high grade cervical neoplasias (n=161)	<ul style="list-style-type: none"> HPV clearance of viral DNA from early precancer lesions 81.3.% HPV clearance rate in women treated with curcumin vaginal capsules No serious adverse events were noted 	(195)
Curcumin	Targets oncogene expression, anti-cancer	<i>In vitro</i> / Biopsies	HeLa	<ul style="list-style-type: none"> Downregulated HPV18 transcription selectively downregulate viral transcription by targeting AP-1 activity 	(44)
	Targets oncogene expression, anti-cancer	<i>In vitro</i>	HeLa, SiHa, CaSki, C33a	<ul style="list-style-type: none"> inhibited expression of viral oncogenes E6 and E7 Inhibition through abrogation of AP-1 and NF-kB activity Antiproliferative and induced apoptosis 	(189)
	Targets oncogene expression, anti-cancer	<i>In vitro</i>	SiHa, C33a, 93UV147T	<ul style="list-style-type: none"> Inhibit expression of viral oncogenes E6 and E7 Block constitutively active STAT3, NF-kB, AP-1 and expression of their oncogenic constituent members 	(45, 267, 374)
Curcumin Analogue 1,5-Bis (2-hydroxyphenyl)-1,4-pentadiene-3-one	Targets oncogene expression, anti-cancer	<i>In vitro</i>	HeLa and CaSki	<ul style="list-style-type: none"> Cytotoxic and anti-proliferative activity Down-regulated HPV18- and HPV16-associated E6 and E7 oncogene expression 	(255)
Berberine	Targets oncogene expression, anti-cancer mechanism	<i>In vitro</i>	HeLa, SiHa and C33a	<ul style="list-style-type: none"> Inhibited AP-1 transcription factor and blocked viral oncoproteins E6 and E7 expression Reduce the cell viability through mitochondria-mediated pathway by activating caspase-3 Increase p53 and Rb expression and suppressed expression of hTERT 	(52)
	Anti-viral	<i>In vitro</i>	HeLa, C33a	<ul style="list-style-type: none"> Modulates HPV-18 E6–E7 oncoproteins by targeting p53 	(196)
Nordihydroguaiaretic acid (NGDA) from plant lignin	Targets oncogene expression, anti-cancer mechanism	<i>In vitro</i>	Recombinant Vector Constructs	<ul style="list-style-type: none"> Inhibition of HPV16 transcription by blocking SP1-mediated transcription Stabilization of p53 	(199, 200)
Jaceosidin (4',5,7-trihydroxy-3',6-dimethoxyflavone) (Isolated from <i>Artemisia argyi</i>)	Targets oncogene function, anti-cancer mechanism	<i>In vitro</i>	SiHa and CaSki	<ul style="list-style-type: none"> Inhibit binding of E6 with p53 and E7 with pRb Suppress growth of cervical cancer cells 	(197)
Wogonin	Targets oncogene expression, anti-cancer mechanism	<i>In vitro</i>	CaSki, SiHa and C33a	<ul style="list-style-type: none"> Induce apoptosis mediated by inhibiting E6/ E7 expression Inducing intrinsic pathways in cervical cancer 	(198)
Other natural products					
Heparin	Targets oncogene expression, anti-cancer	<i>In vitro/ in vivo</i>	HeLa	<ul style="list-style-type: none"> Inhibition of AP-1 activity and inhibition of HPV LCR and transcription of E6 & E7 Suppression of cell proliferation, G2/M cell cycle arrest. 	(201)

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Carrageenan (Polysaccharide extracted from marine red algae (seaweed))	Blocking viral entry, immunogenic	<i>In vitro</i>	HeLa	• Acts primarily by preventing the binding of HPV virions to cells	(202)
	Blocking viral entry	<i>In vivo</i>	Mouse model of cervico- vaginal infection with HPV16 pseudovirus transduction of a reporter gene	• Prevented the establishment phase of HPV infection	(212)
	Blocking viral entry	Preclinical	Female rhesus macaques treatment (n = 8) or without (n = 4) infected with HPV16 pseudovirus transduction with a reporter gene	• Decreased mean number of HPV infectious events	(375)
Sulindac (NSAID)	Anti-viral and Anti- cervical	<i>In vitro</i>	HeLa	• Degradation of E7 by the proteasomal pathway • Inhibition of host cell cycle and suppression of cyclin E and A, inactivation of cdk2 • Strong inhibition of the G1 to S transition	(376)
Sulfated K5 E. coli polysaccharide derivatives	Blocking viral entry	<i>In vitro</i>	SiHa and CaSki	• Prevented the interaction between HPV-16 pseudovirions and immobilized heparin • Inhibited HPV-16, HPV-18, and HPV-6 pseudovirion infection	(203)
Plant extracts					
Pinellia pedatisecta Schott (PE) (Rhizome extract)	Targets oncogene expression, anti- cancer	<i>In vitro</i>	CaSki and HeLa	• Down-regulate E6 gene expression at both mRNA and protein levels • Activated p53 stabilizaion and induced apoptosis • Inhibit cell proliferation	(204)
Bryophyllum pinnata (B. pinnata) (Fractionated leaf extract)	Targets oncogene expression, anti- cancer	<i>In vitro</i>	HeLa	• A specific anti-HPV activity on cervical cancer cells as evidenced by downregulation of constitutively active AP1 specific DNA binding activity • Inhibition of HPV18 transcription • Suppression of the anti-apoptotic molecules Bcl-2, and activation of caspase-3	(205)
Phyllanthus emblica Linn. (Crude fruit extract)	Targets oncogene expression, anti- cancer	<i>In vitro</i>	HeLa, SiHa	• Suppression of viral transcription • Induction of apoptotic cell death • Inhibitor of viral entry of HPV16 pseudovirion in cervical cells	(207)
Brucea javanica oil emulsion (BJOE)	Targets oncogene expression, anti- cancer	<i>In vitro</i>	CaSki	• Down-regulating expressions of HPV16 E6 and E7 oncogenes • Induced apoptosis	(206)
Formulations based on herbals and phytochemicals					
Praneem cream (Leaf extract of Azadirachta indica, Saponins, purified extracts of Emblica officinalis, Mentha citrata oil, and extracts of Aloe vera gel)	Elimination of HPV DNA/ HPV-infected cells	<i>In vitro</i> and Phase I/II	Women positive for HPV16 infection without or with low grade squamous intraepithelial lesion (LSIL) or inflammation (n = 20) intra-vaginal application for 30 days	• Clearance of viral DNA from precancer lesions • Elimination of highly carcinogenic HPV type 16 from the uterine cervix	(208)

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Basant cream (Curcumin, Purified saponins, Extracts of Emblica officinalis, Mentha citrate oil and Extracts of Aloe Vera gel)	Inhibited viral transduction	<i>In vitro</i> HPV transduction and Rabbit vaginal application	Bacterial and fungal isolates, HIV-1NL4.3. in CEM-GFP reporter T and P4 (Hela-CD4-LTR-betaGal) cell lines, Rabbit vaginal applications for toxicology	<ul style="list-style-type: none"> Inhibited the growth of Neisseria gonorrhoeae, Candida glabrata, Candida albicans and Candida tropicalis isolated from women with vulvovaginal candidiasis Displayed a high virucidal action against HIV and HPV EC50 (effective concentration 50%) of 1:20000 dilution and nearly complete (98–99%) inhibition at 1:1000 dilution. Two ingredients, Aloe and Amla, inhibited the transduction of human papillomavirus type 16 (HPV-16) pseudovirus in HeLa cells at concentrations far below those that are cytotoxic and those used in the formulation. Found safe in pre-clinical toxicology carried out on rabbit vagina after application for 7 consecutive days or twice daily for 3 weeks. 	(210)
	Elimination of HPV DNA/ HPV-infected cells	Phase I/ II, placebo controlled	HPV positive women without high grade cervical neoplasias (n=126)	<ul style="list-style-type: none"> HPV clearance rate in Basant arm (87.7.%) was significantly higher than the combined placebo arms (73.3.%). Vaginal irritation and itching, mostly mild to moderate after Basant application. 	(195)
	Elimination of HPV DNA/ HPV-infected cells	phase I/III	HPV positive women without high grade cervical neoplasias (n=11)	<ul style="list-style-type: none"> HPV Clearance of viral DNA from precancer lesions HPV clearance rate 100% 	(211)
MZCL-IVR (Core-matrix intravaginal contains MIV-150 (targets HIV-1), zinc acetate (ZA; targets HIV-1 and HSV-2), carrageenan (CG; targets HPV and HSV-2), and levonorgestrel (LNG; targets unintended pregnancy)	Blocking viral entry	<i>In vitro</i> and <i>in vivo</i>	HeLa; Macaques (n = 33)	<ul style="list-style-type: none"> Release testing showed controlled drug release for 94 days <i>in vitro</i> and up to 28 d (study period) in macaques. Showed anti-viral activity in pseudovirion assay with IC50 of 22.8. and 11.5. ng/ml alone and in combination with other drugs respectively 	(213)

through downregulation of essentially required transcription factors AP-1, NF- κ B and STAT3 activity by curcumin (44, 45, 189). Blocking of HPV transcription was accompanied with induction of apoptotic cell death in cervical cancer cells. The effect was observed in HPV-positive cancer cells irrespective of the infecting high risk HPV types. Subsequent studies that evaluate anti-cancer activities of curcumin in cervical cancer cells also validated these findings (191, 192). A preliminary testing of oral administration of curcumin upto 12gm a day for 3 months in 4 CIN cases demonstrated clinical safety of the phytochemical with histological improvements in 1 out of 4 cases (193). Later, phase I/II double-blind clinical trial were conducted using intra-vaginal application of curcumin-containing gelatin pessaries in women infected with HPV and without any high grade CIN (194). The study showed higher clearance of cervical HPV infection (81.3 %) compared to the control arm (73.3 %) (195). The formulation was found safe as no serious adverse events were noted with topical application of curcumin. The major difference among the two studies (193, 194) was the route of administration. Since premalignant tumors in cervix are accessible to topical applications,

any therapy directly applied to the affected tissue appeared to be more effective and avoided the problem of bioavailability that is frequently encountered in working with majority of the phytochemicals when they are administered orally.

Similarly, berberine isolated from perennial herb Berberis displayed anti-HPV activity and suppressed the expression of viral oncoproteins E6 and E7 (52). Berberine possesses anti-inflammatory and anti-cancer properties with no known toxicity. Berberine exerted its inhibitory action through inhibition of host transcription factor AP-1 in a dose- and time-dependent manner and altered the composition of AP-1 DNA-binding complex. Berberine specifically downregulated expression of oncogenic c-Fos which was also absent in the AP-1 binding complex. Loss of E6 and E7 levels was associated with concomitant increase in p53 and pRb expression, loss of telomerase protein, hTERT, and resulted in growth inhibition of cervical cancer cells. Further, berberine altered epigenetic modifications, disrupted microtubule network by targeting p53 in cervical cancer cells (196). Thus, berberine proved its potential as a promising

chemotherapeutic agent in cervical cancer. Though berberine is yet to be tested clinically in cervical cancer it is already being examined in clinical settings for prevention of colorectal adenomas recurrence (NCT02226185). It is also an important component of botanical medical practice including Goldenseal (*Hydrastis canadensis*), Berberis (*Berberis vulgaris*), Oregon grape (*Berberis aquifolium*), Barberry and Chinese Goldthread (*Coptis chinensis*). *Phellodendron chinense* and *Phellodendron amurense* are other two berberine-containing plants that are extensively utilized in Chinese medicine.

Another interesting phytochemical described with anti-HPV activity is Jaceosidin that inhibited the function of E6 and E7 oncogenes by physical interaction with oncoproteins that renders them non-functional (197). Other phytochemicals like Nor-dihydroguaiaretic acid (NGDA) from plant lignin, and wogonin, an anti-cancer flavonoid compound extracted from *Scutellaria baicalensis*, downregulated HPV E6 and E7 expressions and promoted intrinsic apoptosis in human cervical cancer cells through suppression of HPV transcription (198–200). Some other natural products like heparin (201), carrageenan a polysaccharide extracted from marine red algae (seaweed) (202), sulindac (non-steroidal anti-inflammatory drug) and sulfated K5 *E. coli* polysaccharide derivatives (203) reportedly exert anti-viral action against HPV primarily by preventing the entry of the virus to its target cells. Apart from the purified chemical entities, some crude and partially fractionated extracts of medicinal plants like rhizome extract of *Pinellia pedatisecta* Schott, Fractionated leaf extract of *Bryophyllum pinnata* rich in Bryophyllin A, crude fruit extract of *Phyllanthus emblica* Linn. and *Brucea javanica* oil emulsion has shown potent anti-HPV activities that are mediated through their inhibitory action on host factors like AP-1 and STAT3. These transcription factors promote cervical carcinogenesis and specifically downregulated expression of viral oncogenes E6 and E7 (204–207) (Table 4).

Based on safety of herbal ingredients and their general anti-microbicidal action, a few poly-herbal formulations or mixtures based on herbal derivatives have been evaluated in clinical studies. Praneem, a combination of leaf extract of *Azadirachta indica*, saponins, purified extracts of *Emblica officinalis*, *Mentha citrata* oil, and extracts of *Aloe vera* gel was examined in HPV infected women in a placebo controlled clinical study (208). One course of thirty day intra-vaginal application of Praneem polyherbal tablet in women with early cervical intraepithelial lesions resulted in elimination of HPV16 infection in 60% of the women (6/10). The patients that could not clear HPV infection were subjected to another round of Praneem treatment and HPV clearance was observed in 50% of individuals resulting in total of 80% HPV clearance rate

(208). Though the mechanism of action of Praneem or its individual components is not known, but a general microbicidal activity of the formulation against reproductive tract infections that can promote HPV-mediated cervical carcinogenesis as co-factors had been reported earlier (209). Later, following discovery of curcumin's specific action on HPV transcription (44), another polyherbal cream formulation was developed and named 'Basant' for its saffron color that was imparted by chief ingredient curcumin. The formulation apart from curcumin, contained purified saponins, extracts of *Emblica officinalis*, *Mentha citrata* oil and extracts of *Aloe vera* gel that were common with Praneem (210). Although Basant showed a broad spectrum anti-microbicidal activity against drug resistant bacterial and fungal strains, it had a strong virucidal activity against HPV as well as HIV in *in vitro* analysis. Two of its ingredients, Aloe and Amla, which also formed part of Praneem, inhibited the transduction of HPV16 pseudovirus in HeLa cells at concentrations far below those that are cytotoxic and those used in the formulation. Therefore, the mechanism of these two components may be different and complementary to curcumin which target viral transcription (44, 45). Though Basant was found to be totally safe according to pre-clinical toxicology carried out on rabbit vagina by applying the formulation for 7 consecutive days or twice daily for 3 weeks (210), in clinical studies a mild to moderate vaginal irritation and itching was reported after Basant application (195). In this double blind randomized clinical evaluation study, women showed a statistically significant clearance of HPV infection following Basant application (87.7 % vs. placebo arms -73.3 %) (195). In another recent clinical study, Basant application in 11 women infected with HPV with low grade cervical abnormalities resulted in clearance of HPV16 infection in all the women recruited (11/11) (211). Further large scale clinical evaluation of these promising polyherbals is warranted to make them a useful, effective, cheap and clinically available therapeutic.

Apart from herbal derivatives, other natural sources have been explored for potential anti-HPV activity. The most prominent among them was carrageenan which was found to be is a potent inhibitor of HPV infection (202). Carrageenan extracted from marine red algae is composed of a sulfated polysaccharides of D-galactose and 3, 6-anhydro-D-galactose, and present in some vaginal lubricants (184). Carrageenan prevented the entry of the virus into the host cell both *in vitro* (202) and in animal models (212). A novel intra-vaginal ring of core-matrix having combination microbicide containing carrageenan as constituent to prevent HPV infection have been developed (213). Further clinical trials are needed to determine the efficacy of carrageenan-based products against genital HPVs in humans. Similarly, alginic acid and fucoidan derived from

red algae were found to possess anti-HPV infection activities in high-throughput *in vitro* screens based on high-titer HPV pseudoviruses to identify HPV infection inhibitors (202). Other marine derivatives like gliotoxin, neoechinulin A, physcion, epoxydon and heterofucan also showed generic anti-cervical cancer activity and have been reviewed separately (184). Further studies are needed to examine their anti-HPV activities.

In view of the emerging anticancer activities, the herbal derivatives are being examined for their use as adjuvant to mainstream chemo- and radiotherapies for treatment of cervical cancer. Preliminary investigations demonstrate potential role of phytochemicals as chemosensitizers in cervical cancer. Phytochemicals like curcumin sensitized cervical cancer cells to taxol-induced or cisplatin-induced apoptosis by down-regulation of NF- κ B (214–216). Similarly, other phytochemicals like quercetin, saikosaponins, wogonin, tea polyphenol epigallocatechin gallate (EGCG) and apigenin also sensitized cervical cancer cells to cisplatin (217–220), resveratrol, trihydroxyisoflavone and formononetin were found to sensitize cervical cancer cells to different anthracyclines (217–220). Further, dietary flavonoids sensitized cervical cancer cells to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) (221). TRAIL is another promising candidate as cancer therapeutics that preferentially induces apoptosis in cancer cells. Flavone, apigenin, genistein, decursin and decursinol markedly augmented TRAIL-mediated cytotoxicity (221, 222). Many of these phytochemicals mediate their action through reactive oxygen species (ROS) (218–221, 223–226) or targeting different drug resistance mechanisms upregulated by viral oncogenes (51, 158).

A few phytochemicals have shown potent radiosensitizing effects. Resveratrol (227), genistein (228–230), curcumin (223, 231), ferulic acid (232) and quercetin (233) have shown synergistic anti-cancer properties and potentiation of apoptotic effects of ionizing radiation on cervical cancer cells primarily *in vitro* and in some studies *in vivo* (233). A ROS-dependent mechanism has again been implicated in radiosensitivity mediated by at least some of the phytochemicals (223).

With evolution of sophisticated molecular modeling, *in silico* approaches have been utilized to screen phytochemicals for their anti-HPV activity by specifically targeting molecular interaction of viral oncogenes with their respective cellular targets, thus rendering the viral oncogenes non-functional (234–237). *In silico* docking of Carrageenan, Daphnoretin, Withaferin A and Artemisinin against HPV16 and HPV18 E6 revealed nearly 20X stronger inhibitory effect as compared to curcumin and tea polyphenol EGCG or jaceosidin found to functionally interfere

with E6 (234–236). Further studies are needed to validate these findings in experimental settings. Nevertheless, the approach is extremely powerful tool to examine potentially useful phytochemicals and developing them into a rationally-designed drug in a very short period.

Pure herbal derivatives, even though show remarkable anti-cervical cancer or anti-HPV activity in *in vitro* studies, they have poor bioavailability due to factors that vary with each derivative. Solubility of herbal derivatives in water has been a great challenge which prevents their intravenous administration. Therefore, majority of the herbal derivatives are administered orally, and it is the foremost reason of reduced bioavailability which is attributed to poor absorption, and metabolism while in gut or in liver followed by rapid excretion through kidney. These plant products are metabolized by hydrolysis, glucuronidation or glycosidation by gut flora [reviewed in (238)]. Such modifications severely impact the activity of those plant products where the metabolites are inactive. It is, therefore, important that these herbal derivatives be used in combination with biomolecules like piperine that is known to prevent glucuronidation (239–241). Further, mixing of two or more herbal products essentially requires optimization of mixtures which further increases magnitude of *in vivo* experimentation. Alternatively, nano-formulations and chemically-modified herbal derivatives have been examined in cervical cancer cells and have shown superior anti-cancer effects compared to their native counterparts at an equal strength in some cases. During last decade, there has been a significant progress in nano-formulations and derivatization of phytochemicals to improve their efficacy and bioavailability. Experimental combination of natural products/phytochemicals with silver nano- or liposomal formulations have been attempted to improve the anti-cancer activity (242–245) or to improve the chemosensitization of cervical cancer cells (246, 247). Phytochemicals derivatized to enhance activities against cervical cancer cells include lignin, chrysin, nitroflavone, propyl gallate, bromofascaplysin, butylated hydroxyanisole, flavonoid and curcumin (248–255). Treatment with most of the chemically-modified derivatives resulted in reduced proliferation and enhanced apoptosis and showed targeting of different but complementary anti-cancer mechanisms such as inhibiting telomerase and activation of c-myc and caspases by substituted methylenedioxy lignan (254), or cell cycle arrest G2/M by nitroflavone (249) and in G1 phase by propyl gallate (250), or GSH level depletion by butylated hydroxyanisole (252) or recently by downregulation of HR-HPV E6 and E7 (255).

Apart from chemically-defined entities or their nano-formulations, all other plant derivatives suffer from common drawbacks of batch to batch variation. Nevertheless, the herbal derivatives' group as a

whole shares many advantages include abundance, ease of isolation and their pharmacological safety that make them low cost, effective therapeutic entities. Despite these advantages, highly encouraging leads obtained from herbal or natural sources are not being put forward in large scale Phase II/III/IV clinical trials and preventing these formulations to become a clinical reality. Because of prevailing stringent regulations, execution of interventional clinical trials is now becoming more and more expensive, labor-intensive and demands an exhaustive infrastructure. Unfortunately, due to low commercial gains associated with these commonly-used, widely-available traditional and herbal remedies, testing these entities in large-scale clinical trials have become prohibitive in itself thus creating a major bottleneck and a roadblock to introduction of anti-HPV molecules/formulations from herbal sources in mainstream medicine.

6.5. Anti-HPV leads in alternative medicines

Interestingly, there are several parallel and concurrent systems of medicine that are operating along with mainstream medicine which extensively utilize same plants as source of their medicines. Among them, Indian, Chinese, Japanese and many other traditional and folklore medicines are popularly being practiced along with mainstream allopathic medicine all over the world. Nevertheless, Homeopathy is one of the most accepted 2nd line clinical management systems operating globally. Several formulations are well-standardized, and commercially available. Formulations are available for the treatment of symptoms associated with genital warts and cervical cancer these include Iodum, Kreosotum, Natrum Carb, Carbo Animalis, Calcaria Fluor, Hydrocotyle, Sabina, Calcaria Carb, Kali Iodide, Conium, Hydrastis, Sanguinaria, and Lachesis (256). Despite claimed efficacy, the mechanism of action of these homeopathic formulations/drugs, particularly on HPV and its oncogenes, is not known. Only a few reports describe the anti-cancer effect of homeopathic remedies and their mechanism of action in experimental cancers and cell cultures (257–260). On the other hand, Lycopodium from *Lycopodium clavatum* extract has long been used in complementary and alternative medicine for treating various liver ailments and Alzheimer's disease, was found to inhibit proliferation of HeLa cells and induced caspase-3 mediated apoptosis (261). Similarly, *Conium maculatum* extract which is used as a traditional medicine for cervix carcinoma including homeopathy, induced apoptosis in cervical cells through ROS generation (262). Drug-DNA interaction was implicated in this phenomenon. Interestingly, recent studies showed existence of nanoparticle-like structures in some homeopathy formulations (239, 263–265). In this regard, it would be relevant

to investigate anti-HPV effects and mechanism of action of these homeopathic drugs. Alternatively, as a second approach, the homeopathic remedies that utilize herbal derivatives outlines above (curcumin, berberine, Bryophyllum, Phyllanthus, spindus, Azadirachta etc.) may be explored for potential anti-HPV activities. These research objectives should be currently the area of active investigation for discovery and development of low-cost anti-HPV molecule (s).

7. CONCLUSION

It is a matter of prime importance to develop anti-HPV molecules/formulations that could eliminate HPV infection and are safe. Since HPV and related malignancies are multifactorial with long incubation period of the virus, combination of approaches may be used for effective control of virus-driven oncogenic progression. Targeting HPV persistence and specific downregulation of viral oncoproteins by any HPV-specific approach whether using therapeutic vaccines or genome silencing or genome editing are expected to provide a useful therapy. However, in practice such approaches have never been foolproof and rewarding and the reason could be that some of the malignant lesions despite HPV positivity behave like an HPV-negative tumors at molecular level. Recent TCGA (The Cancer Genome Atlas) study examined genome features of cervical cancers and identified novel genomic and molecular characteristics of HPV negative endometrial-like cancers (266). Therefore, it is important that investigational drugs under development not only target HPV or its oncogenes but also additionally target factors that influence carcinogenic progression; which could also curtail local inflammation by eradicating co-infectors and dampening of carcinogenic pro-inflammatory transcription factors that additionally activate HPV transcription. Incidentally, phytochemicals and some of the plant derivatives have been shown to target such carcinogenic transcription factors (43–45, 50–52, 158, 189, 205, 207, 208, 267) and this area should be explored further more vigorously. Further, developing active principles and leads from alternate medicine that already have well-defined safety and efficacy profile, as potential anti-HPV therapeutics against cervical cancer in mainstream medicine will be economic, easily translatable in clinic without undergoing expansive phase II/III/IV clinical trials and will help in creation of a fusion medicine.

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Abbreviations: AS-ODNs – antisense oligonucleotides; CIN – cervical intraepithelial neoplasia; CRISPR/Cas9 – clustered regularly interspaced short palindromic repeat-associated nuclease cas9 RNA-guided endonuclease; DSB – double-strand breaks; Dz– DNazymes; EBRT – external beam radiation therapy; gRNA – guideRNA; HPV – human papillomavirus; HR – high-risk; LEEP – loop electrosurgical excision procedure; LR – low-risk; NA– nucleic acid; pRB– retinoblastoma protein; RNAi– RNA interference; ROS – reactive oxygen species; Rz– ribozymes; shRNA– short hairpin RNA; siRNA– short interfering RNA; TALEN– transcription activator-like effector nucleases; TRAIL – tumor necrosis factor-related apoptosis-inducing ligand; Tregs – T regulatory cells; US-FDA– United States Food and Drug Administration; VIA – visual inspection with acetic acid; VIA –visual inspection with Lugol’s iodine; ZFN – zinc finger nucleases

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