The Development of Therapeutic Vaccines to Treat HPV-medicated Cervical Cancers

Emma Machuik, Simon Fraser University

Keywords: HPV-mediated cervical cancer, therapeutic vaccines, vaccine development

SFU Health Sciences Undergraduate Journal

This literature review investigates the development of therapeutic vaccines to treat HPV-mediated cervical cancers. Specifically, HPV-16 targeting vaccines are reviewed. Vaccines are grouped by type: vector-based, DNA-based, synthetic plasmids, and long peptide vaccines. The review is based on study endpoints, tolerability, safety, and efficacy. Commonalities seen throughout all studies were the use of E6 and E7 proteins as targets, IFNy response measures, and regression as a marker of efficacy. Each vaccination against HPV-16 mediated cervical cancer showed tolerability and safety. However, they did not all show adequate efficacy. The two DNA/RNA-based vaccinations that showed the most promising results were VB10.16 and VGX-3100. This indicates that further research is needed within this field.

Cancer in Canada

Ask any student who grew up in Canada if they remember their childhood immunizations and nearly everyone will laugh and detail experiences getting their various vaccines at school surrounded by friends. One of these is the human papillomavirus (HPV) vaccine. What many fail to recognize is how important these vaccines are and how lucky we are to have access to a prophylactic vaccine for HPV. Four prophylactic vaccines are currently approved for use: Cervarix, Gardasil, Gardasil-9, and Cecolin (Akhatova et al., 2022). In 2019, it was estimated that global HPV immunization coverage (full course of vaccines) was 15% of girls and 5% of boys (Bruni et al., 2021; Spayne & Hesketh, 2021). If not immunized, it is estimated that 75% of sexually active Canadians will experience an asymptomatic HPV infection in their lifetime (*Human Papillomavirus (HPV) Vaccines: Canadian Immunization Guide - Canada.Ca*, n.d.). HPV is associated with a plethora of medical concerns, from lesions and warts to cancer (Muñoz et al., 2003).

HPV is the leading cause of cervical cancer in women, also causing approximately 90% of anal, 70% of vagina, 50% of penile, and 40% of vulvar cancers (Garland et al., 2016). The development of a therapeutic vaccine to treat cervical cancers caused by HPV has been an area of research for many years, and many advancements have been made. This paper investigates these developments and evaluates various vaccines intended to treat cervical cancers associated with HPV-16.

Human Papilloma Virus

Disease Etiology and Pathogenesis

HPV infections are sexually transmitted diseases that can be seen in both men and women. HPV is the most common sexually transmitted virus worldwide (Garland et al., 2016). HPV originates from the papillomavirus, which can infect humans through basal epithelial cells (Burd, 2003). There are two types of infection based on the route of the infection. Cutaneous type is infectious through the skin on the hands and feet (Burd, 2003). Mucosal type infects humans through the lining of the mouth, throat, respiratory tract, anus, or genital epithelium (Burd, 2003).

Cervical cancer develops from squamous intraepithelial lesions created by HPV (see Figure 1 in appendix) (Knoff et al., 2014). Cervical cancer is one of the leading causes of death in women, and the fourth most common cancer in women around the globe. (*Cervical Cancer: A Global Health Crisis - Small - 2017 - Cancer - Wiley Online Library*, n.d.). HPV can be separated into high-risk and low-risk based on the cancers that originate from them. High-risk HPV are types 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 70 (Burd, 2003). Of these high-risk types, HPV 16 and 18 are responsible for 70% of cervical cancers seen today

(Spayne & Hesketh, 2021). This is why they are important targets for the treatment of cervical cancer and, thus, why HPV-16 will be the focus of this review.

Review of the Literature: Therapeutic Vaccines and Cancer Development

Vector-based Vaccines

Virus-based

The first vaccine examined is the RNA virus-based viral vector vaccine, Vvax001. It is made of replication-incompetent Semliki Forest virus (SFV) replicon particles, which encode for E6 and E7 antigens from HPV-16 (Komdeur et al., 2021). In this clinical trial, 12 participants with cervical intraepithelial neoplasia grade 2 and 3 (CIN II/III) were designated to three cohorts of four participants. Each cohort received a varied dose of Vvax001: 5×10^5, 5×10^6, 5×10^7, and 2.5×10^8 infectious particles per immunization. Each group was immunized three times with three-week intervals between immunizations. Adverse events (AEs), interferon-gamma (IFNy), and T-cell responses were measured. All four dose levels had mild AEs, such as chills, injection site hematoma, and back pain. However, no serious adverse events were reported. Also, there was no correlation between adverse events and vaccination dose, indicating no dose-related toxicity.

ELISPOT was used to measure the presence of IFN-y-producing cells using peripheral blood mononuclear cells (PBMC) isolated from blood samples pre- and post-vaccination to measure T-cell response (Komdeur et al., 2021). Every dose cohort had a vaccine-induced T-cell response, with 83% of participants seeing a statistically significant HPV-16 E6/E7 response. The response against E6 was stronger than the responses to E7. Overall, this study showed promising results for virus-based therapeutic vaccines for HPV-mediated cervical cancer, specifically

regarding safety and tolerability factors. However, the small sample population limits the generalizability of these results, and thus, further research must be done on this vaccine.

Bacteria-based

Lm-LLO-E7 is a live-attenuated Listeria vaccine designed to treat patients with CIN II/III (Maciag et al., 2009). This study was a phase I safety study to evaluate the safety and feasibility of a bacterial-based vaccine to treat HPV-mediated CIN II/III (Maciag et al., 2009). Fifteen participants were separated into three dosing levels and received vaccination intravenously twice with a three-week separation period. The primary outcome of this study was adverse events, and exploratory outcomes were tumour size regression and the survival of the participants. All patients experienced some form of adverse events, with 100% of patients experiencing a rise in body temperature, 60% of patients experiencing vomiting, and 53.3% experiencing chills, headache, and anemia. There were no serious adverse events or deaths associated with the vaccination. Tumour size regression was seen in 7.7% of patients, with 53.8% having stable disease and 38.5% having progressive disease upon follow-up. There was no dose response seen between dose level and tumour regression.

Overall, this study demonstrated the relative safety of listeria-based vaccination for HPV-mediate cervical cancer. However, the relative numbers of adverse events and low regression rates indicate that other types of vaccines may be more effective for treating this disease.

Peptide and Protein Based Vaccines

The next type of vaccines investigated were peptide and protein-based vaccines. The vaccine study used to investigate this branch of vaccines is a long peptide vaccine containing 13 peptides which encoded HPV-16 E6 and E7 proteins within a Montanide ISA-51 adjuvant (Kenter et al., 2008). Within this study, 35 participants with CIN II/III were separated into three

cohorts; each cohort received a different dose of vaccine. Cohort 1 received 300 ug of the long peptide at one injection site, cohort 2 received 100 ug of long peptides E6 in one arm and 300 ug of long peptides E7 in the other arm, and finally, cohort 3 received two different injections at 50 ug of long peptides E6 and E7 respectively. The outcomes measured were safety and immunogenicity. There were no serious adverse effects related to vaccination. Mild adverse effects were seen in all participants who rated the vaccination as mildly painful; 8.6% reported mild fevers (below 40 °C), and 6.1% reported flu-like symptoms.

Immunogenicity was measured using PMBC from blood taken before and after vaccination, ELISPOT was used to measure IFNy producing cells to determine if an HPV-16 specific T-cell response was mounted (Kenter et al., 2008). In cohort 1, 100% of participants had HPV-16 E6 specific T-cell responses, and 57.1% had HPV-16 E7 specific responses. In cohort 2, 81.8% of participants had E6 specific T-cell responses and 63.6% had E7 specific T-cell responses. In cohort 3, 66.7% of participants responded to E6, and 44.4% responded to E7. Overall, this study supports the use of long peptides to produce immunogenicity against HPV-related cervical cancer. However, a lack of investigation into the effect of lesion regression is seen, which lowers the applicability of results to clinical settings. Further research surrounding this area is needed.

DNA and RNA Based Vaccines

The next type of vaccines reviewed are DNA and RNA based Vaccines. The first vaccine in this type is VB10.16, an antigen-presenting cell (APC) targeting, DNA based vaccine (Hillemanns et al., 2022). Similarly to other vaccines reviewed, it targets HPV-16 E6 and E7 proteins. This vaccine is formed by taking inactivated versions of these antigens and linking them with a human chemokine motif ligand, forming a dimer (Hillemanns et al., 2022). When a

patient is immunized, this chemokine will attract APCs, activating specific T-cells to mount a specific T-cell response to the HPV-mediated cancer, such as high-grade CIN (Hillemanns et al., 2022).

This study was an open-label phase I/II clinical trial with 34 participants, all women with HPV-16 mediated CIN II/III (Hillemanns et al., 2022). Two initial dosing cohorts of eight participants were used to determine the efficient dosing schedule; both cohorts received 3 mg vaccinations of VB10.16. Cohort 1 received vaccinations at 0, 3, and 6 weeks, while Cohort 2 received vaccinations at 0, 4, and 12 weeks. Following this assessment, an expansion cohort of 18 participants was created following the dosing used in cohort 2. To determine the safety, immunogenicity, and efficacy of VB10.16, the primary endpoints in this study were adverse events, E6/E7 specific cellular response, HPV-16 clearance, and regression of CIN lesion. No serious adverse effects were seen. However, mild adverse effects were present in 81% of participants, such as injection site reactions, headache, and erythema.

A reduction in lesion size and grade measured clinical efficacy. In the expansion cohort, 71% of participants had a reduction of lesion size greater than 50% compared to the baseline (Hillemanns et al., 2022). Meanwhile, 75% of patients in the initial dosing of Cohort 1 and 50% of Cohort 2 saw a reduction of 50% or greater. A regression from CIN 0 or I was seen in 59% of participants, and complete regression was seen in 47% of participants within the expansion cohort. Within the initial dosing cohort, 38% of Cohorts 1 and 2 participants saw a regression to CIN 0 or 1, and 25% of Cohorts 1 and 2 saw complete regression.

HPV-16 clearance was measured using immunohistochemistry and CobasHPV testing. In the expansion cohort, 47% of patients achieve clearance. While in the initial dosing cohort, 38% in cohorts 1 and 2 saw HPV-16 clearance (Hillemanns et al., 2022). Specific T-cell responses

were measured using ELISPOT IFNy assay using PBMC from blood taken pre- and post-vaccination. In the expansion cohort, specific T-cell responses were seen in 94% of participants; this response was seen against both antigens (Hillemanns et al., 2022). In the initial dosing cohort, Cohort 1 responded in 84% of participants, while Cohort 2 responded in 100%. Between all cohorts, 79% of participants saw a reduction in lesion size that statistically correlated with the strength of their T-cell responses ($P>0.001$). Overall, this vaccine demonstrated good tolerability. The addition of HPV-16 clearance and tumour regression strengthens the results' implications despite the relatively small sample size.

The next vaccine evaluated was VGX-3100, a synthetic DNA vaccine (Trimble et al., 2015). This vaccine is made of synthetic plasmids which encode E6 and E7 of HPV-16 and HPV-18, respectively. This study was a randomized, double-blind, placebo-controlled phase IIb clinical trial (Trimble et al., 2015). There were 167 participants, all of whom had CIN II/III associated with HPV-16. Participants were randomized to control (n=42) and experimental cohorts (n=125) with stratification based on age and severity of CIN. The experimental cohort received a 6 mg dose of VGX-3100, which contained 3 mg of plasmids, while the control cohort received an injection of sterile saline. Vaccinations were done at 3, 4, and 12 weeks, while assessments took place two weeks after each dose. The outcomes measured were adverse events and efficacy.

No serious adverse effects were seen in either cohort (Trimble et al., 2015). Mild adverse events were seen in the experimental cohort, such as injection-site reactions, fatigue, headache, and nausea. Interestingly, tolerability was the same for both experimental and placebo cohorts. Efficacy was measured as a regression of CIN lesions to CIN I or normal pathology up to 36 weeks after the initial dose. A secondary efficacy measure was HPV-16 and HPV-18 clearance.

Regression was seen in 49.5% of experimental participants compared to 30.6% of placebo participants. In comparison, HPV clearance was seen in 40.2% of experimental participants and 14.3% of placebo participants.

This study was known as a landmark study as it was the first of its kind to meet primary endpoints (Yan et al., 2023). Its strong sample size and the inclusion of multiple endpoints allowed for the collection of maximal data, thus allowing for further data analysis and conclusions.

Discussion

Use of E6 and E7 Proteins as Targets

A common thread in each of the vaccines investigated is the use of E6 and E7 proteins as targets for their mechanism of action. These proteins are believed to drive cell proliferation (Rumfield et al., 2020). This is due to the dysregulation seen in early cancer proliferation of early protein 2, which acts as a regulator for the expression of E6 and E7; when E2 is repressed, there is an overexpression of these two proteins, which results in mass cell proliferation. E7 destroys retinoblastoma (Rb) proteins, which act as a repressor in cell proliferation; E6 destroys p53, which similarly repressed cell proliferation (Knoff et al., 2014; Rumfield et al., 2020).

Using these E6 and E7 proteins as targets benefits vaccine design as they are expressed highly in the malignant cells and not as highly expressed among host cells (Rumfield et al., 2020). Another benefit to using these proteins is that they are an important factor in cancer cell proliferation and transformation (see Table 2 of the appendix) (Knoff et al., 2014). Although this mechanism of action appears to be effective, it is a fault of this paper that other target proteins or mechanisms were not further investigated to produce a more holistic review of current vaccine

development. Current research is being done on targeting other HPV-16 CD4+ epitopes, such as epitopes on E2 and E5 (Grabowska et al., 2015; Yan et al., 2023).

IFNy Response

IFNy responses were measured in most of the studies as a marker of specific T-cell response due to vaccination. This is useful as it is a cost-effective method to obtain a large amount of data (see Table 1 for a summary of all study information and Table 2 4 for a summary of study results). The PBMC from patients is isolated from blood samples and used to isolate T-cells that secrete IFNy when incubated with HPV using detection antibodies (Akache & McCluskie, 2020).

Results from the investigated vaccines showed that VB10.16 (DNA-based vaccine) has the highest IFNy responses in the initial dosing Cohort 2. It should also be noted that they had significantly lower levels of regression and HPV clearance than other experimental groups (Hillemanns et al., 2022). This suggests that this level of IFNy response is not solely indicative of an effective vaccine, as the magnitude and longevity of IFNy response in the initial dosing Cohort 2 was superior. That is why the dosing regimen seen in Cohort 2 was chosen for the expansion cohort, which was far more successful in all outcomes measured (see Table 2). This shows that we must consider the number of participants that mounted a response and how this response was performed.

Kenter et al.'s study on long peptide vaccines also had a strong IFNy response. Interestingly, they delineated their responses between E6 and E7. These results confirmed that E6 mounted a greater IFNy response across all cohorts studied (Kenter et al., 2008). They also found that injection of E6 and E7 peptides in the same location increased the magnitude of the E6 IFNy

response but did not alter the E7 response, confirming no interference when targeting these proteins (Kenter et al., 2008).

The study on Vvax001 (alphavirus-based vaccine) also had a strong IFNy response. This was notable as the lowest dosed cohort at 5 x 10^5 infectious particles per immunization observed a response. Their findings also supported Kenter et al.'s finding that the E6 response was greater than that of E7 (Komdeur et al., 2021).

Regression as a Marker of Efficacy

Regression was also a commonly used marker among the studies synthesized. One of these studies was on VB10.16, which cited that due to the low regression rate of these cervical lesions, a control group was not needed, and therefore, they performed an open-label study (Hillemanns et al., 2022). Interestingly, the VGX-3100 (Synthetic plasmid vaccine) study used a control group and performed a double-blinded study (Trimble et al., 2015). The results from the VGX-3100 showed that the experimental group had a statistically significant number of lesion regressions at 49.5% of participants. However, the placebo group also had 30.6% of participants with lesion reductions (Trimble et al., 2015). This is important to note as a common understanding is that cervical lesions rarely regress without treatment. However, the VGX-3100 study shows that controls are beneficial for understanding the disease and creating a more nuanced study (Trimble et al., 2015).

HPV Clearance as a Marker of Efficacy

Finally, HPV clearance was used in two studies as a secondary outcome. Interestingly, they both found similar clearance levels, sitting at approximately 40% of all patients (Hillemanns et al., 2022; Trimble et al., 2015). The reasoning behind these results is not investigated in either study. However, for HPV clearance to be achieved, a significant regression must be seen, which

was the case with both studies. However, another interesting avenue of investigation would be to evaluate if a lack of HPV clearance plays a role in the reoccurrence of lesions that had previously regressed.

Conclusion

Each of these types of vaccinations against HPV-16 mediated cervical cancer showed tolerability and safety. However, they did not all show adequate efficacy. The two vaccinations that showed the most promising results were VB10.16 and VGX-3100, both DNA/RNA based. This should indicate that further research is needed within this field.

Future research must ensure adequate sample sizes and multiple immunogenicity endpoints are investigated to understand the methods through which these vaccines function fully. Additionally, given the lack of nuanced sex and gender-based analysis in all of the research, its inclusion should be considered for future research in this field. Further research should be performed on other early protein epitopes to determine the most efficient mechanism of action for HPV-16 mediated cervical cancer therapeutic vaccines.

References

Akache, B. & McCluskie, M. J. (2020). Vaccine Delivery Technology, Methods and Protocols. *Methods in Molecular Biology*, *2183*, 525–536. https://doi.org/10.1007/978-1-0716-0795-4_30

Akhatova, A., Azizan, A., Atageldiyeva, K., Ashimkhanova, A., Marat, A., Iztleuov, Y., Suleimenova, A., Shamkeeva, S. & Aimagambetova, G. (2022). Prophylactic Human Papillomavirus Vaccination: From the Origin to the Current State. *Vaccines*, *10*(11), 1912. https://doi.org/10.3390/vaccines10111912

Bruni, L., Saura-Lázaro, A., Montoliu, A., Brotons, M., Alemany, L., Diallo, M. S., Afsar, O. Z., LaMontagne, D. S., Mosina, L., Contreras, M., Velandia-González, M., Pastore, R., Gacic-Dobo, M. & Bloem, P. (2021). HPV vaccination introduction worldwide and WHO and UNICEF estimates of national HPV immunization coverage 2010–2019. *Preventive Medicine*, *144*, 106399. https://doi.org/10.1016/j.ypmed.2020.106399

Burd, E. M. (2003). Human Papillomavirus and Cervical Cancer. *Clinical Microbiology Reviews*, *16*(1), 1–17. https://doi.org/10.1128/cmr.16.1.1-17.2003

Cervical cancer: A global health crisis - Small - 2017 - Cancer - Wiley Online Library. (n.d.). Retrieved December 8, 2023, from https://acsjournals-onlinelibrary-wiley-com.proxy.lib.sfu.ca/doi/10.1002/cncr.30667

Garland, S. M., Kjaer, S. K., Muñoz, N., Block, S. L., Brown, D. R., DiNubile, M. J., Lindsay, B. R., Kuter, B. J., Perez, G., Dominiak-Felden, G., Saah, A. J., Drury, R., Das, R. & Velicer, C. (2016). Impact and Effectiveness of the Quadrivalent Human Papillomavirus Vaccine: A Systematic Review of 10 Years of Real-world Experience. *Clinical Infectious Diseases*, *63*(4), 519–527. https://doi.org/10.1093/cid/ciw354

Grabowska, A. K., Kaufmann, A. M. & Riemer, A. B. (2015). Identification of promiscuous HPV16-derived T helper cell epitopes for therapeutic HPV vaccine design. *International Journal of Cancer*, *136*(1), 212–224. https://doi.org/10.1002/ijc.28968

Hillemanns, P., Denecke, A., Woelber, L., Böhmer, G., Jentschke, M., Schjetne, K. W., Slot, K. M. H. B. & Fredriksen, A. B. (2022). A Therapeutic Antigen-Presenting Cell-Targeting DNA Vaccine VB10.16 in HPV16-Positive High-Grade Cervical Intraepithelial Neoplasia: Results from a Phase I/IIa Trial. *Clinical Cancer Research*, *28*(22), 4885–4892. https://doi.org/10.1158/1078-0432.ccr-22-1927

Human papillomavirus (HPV) vaccines: Canadian Immunization Guide - Canada.ca. (n.d.). Retrieved December 8, 2023, from https://www.canada.ca/en/public-health/services/publications/healthy-living/canadian-immunization-guide-part-4-active-vaccines/page-9-human-papillomavirus-vaccine.html

Kenter, G. G., Welters, M. J. P., Valentijn, A. R. P. M., Löwik, M. J. G., Meer, D. M. A. B. der, Vloon, A. P. G., Drijfhout, J. W., Wafelman, A. R., Oostendorp, J., Fleuren, G. J., Offringa, R., Burg, S. H. van der & Melief, C. J. M. (2008). Phase I Immunotherapeutic Trial with Long Peptides Spanning the E6 and E7 Sequences of High-Risk Human Papillomavirus 16 in End-Stage Cervical Cancer Patients Shows Low Toxicity and Robust Immunogenicity. *Clinical Cancer Research*, *14*(1), 169–177. https://doi.org/10.1158/1078-0432.ccr-07-1881

Knoff, J., Yang, B., Hung, C.-F. & Wu, T.-C. (2014). Cervical Cancer: Development of Targeted Therapies Beyond Molecular Pathogenesis. *Current Obstetrics and Gynecology Reports*, *3*(1), 18–32. https://doi.org/10.1007/s13669-013-0068-1

Komdeur, F. L., Singh, A., Wall, S. van de, Meulenberg, J. J. M., Boerma, A., Hoogeboom, B. N., Paijens, S. T., Oyarce, C., Bruyn, M. de, Schuuring, E., Regts, J., Marra, R., Werner, N., Sluis, J., Zee, A. G. J. van der, Wilschut, J. C., Allersma, D. P., Zanten, C. J. van, Kosterink, J. G. W., … Daemen, T. (2021). First-in-Human Phase I Clinical Trial of an SFV-Based RNA Replicon Cancer Vaccine against HPV-Induced Cancers. *Molecular Therapy*, *29*(2), 611–625. https://doi.org/10.1016/j.ymthe.2020.11.002

Maciag, P. C., Radulovic, S. & Rothman, J. (2009). The first clinical use of a live-attenuated Listeria monocytogenes vaccine: A Phase I safety study of Lm-LLO-E7 in patients with advanced carcinoma of the cervix. *Vaccine*, *27*(30), 3975–3983. https://doi.org/10.1016/j.vaccine.2009.04.041

Muñoz, N., Bosch, F. X., Sanjosé, S. de, Herrero, R., Castellsagué, X., Shah, K. V., Snijders, P. J. F., Meijer, C. J. L. M. & Group, I. A. for R. on C. M. C. C. S. (2003). Epidemiologic Classification of Human Papillomavirus Types Associated with Cervical Cancer. *The New England Journal of Medicine*, *348*(6), 518–527. https://doi.org/10.1056/nejmoa021641

Rumfield, C. S., Roller, N., Pellom, S. T., Schlom, J. & Jochems, C. (2020). Therapeutic Vaccines for HPV-Associated Malignancies. *ImmunoTargets and Therapy*, *9*, 167–200. https://doi.org/10.2147/itt.s273327

Spayne, J. & Hesketh, T. (2021). Estimate of global human papillomavirus vaccination coverage: analysis of country-level indicators. *BMJ Open*, *11*(9), e052016. https://doi.org/10.1136/bmjopen-2021-052016

Trimble, C. L., Morrow, M. P., Kraynyak, K. A., Shen, X., Dallas, M., Yan, J., Edwards, L., Parker, R. L., Denny, L., Giffear, M., Brown, A. S., Marcozzi-Pierce, K., Shah, D., Slager, A. M., Sylvester, A. J., Khan, A., Broderick, K. E., Juba, R. J., Herring, T. A., …

Bagarazzi, M. L. (2015). Safety, efficacy, and immunogenicity of VGX-3100, a therapeutic synthetic DNA vaccine targeting human papillomavirus 16 and 18 E6 and E7 proteins for cervical intraepithelial neoplasia 2/3: a randomised, double-blind, placebo-controlled phase 2b trial. *The Lancet*, *386*(10008), 2078–2088. https://doi.org/10.1016/s0140-6736(15)00239-1

Yan, F., Cowell, L. G., Tomkies, A. & Day, A. T. (2023). Therapeutic Vaccination for HPV-Mediated Cancers. *Current Otorhinolaryngology Reports*, *11*(1), 44–61. https://doi.org/10.1007/s40136-023-00443-8

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.

Appendix

Figure 1

Pathogenesis of HPV Infection to Cervical Cancer

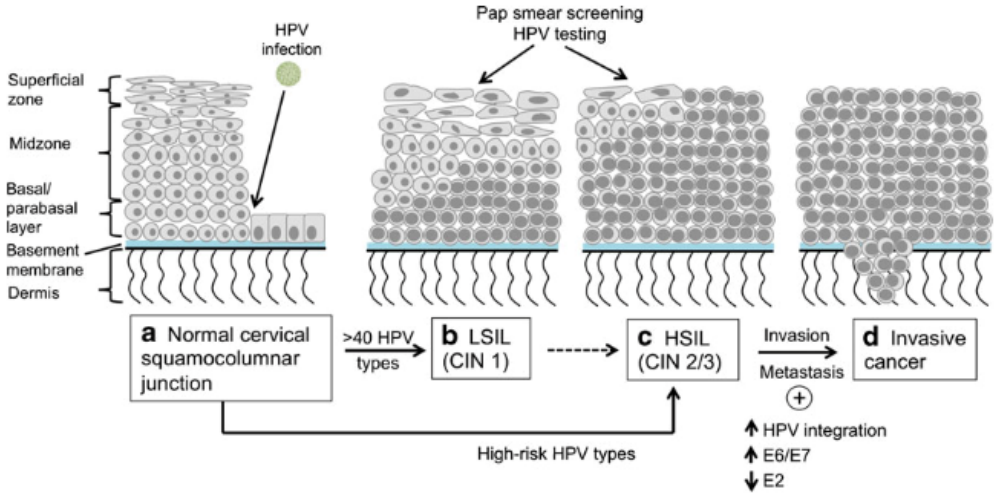

Fig. 1 Cervical squamous intraepithelial lesions (SILs) and HPV-associated pathogenesis. ***b*** The normal cervical squamocolumnar junction. The layer of basal cells that rests on the basement membrane is the normal barrier between the epithelium and the underlying stromal tissue. The parabasal cells form layers of 1–2 cells thick just above the basal cell layer. Normal squamous epithelium differentiates as shown, with the nuclear/cytoplasmic ratio decreasing closer to the surface. The squamocolumnar junction is the most common site for cervical cancer to develop. ***b*** Productive infections produce low-grade squamous intraepithelial lesions (LSILs), in which the basaloid cells occupy the lower third of the epithelium. ***c*** The cancerous precursor pathway is usually initiated by high-risk HPV infections and produces high-grade squamous intraepithelial lesions (HSILs). HSILs show less cellular differentiation, and the basaloid cells occupy at least the lower two-thirds and up to the full thickness of the epithelium. Pap smears and HPV tests can be used to detect SILs. ***d*** If untreated, premalignant lesions can progress into microinvasive or invasive cancer, in which tumor cells breach the basement membrane. This process is associated with integration of the HPV genome into the host chromosomes, loss of E2, and upregulation of viral oncogene expression and genomic instability

Note. The figure "Pathogenesis of HPV Infection to Cervical Cancer" is from "Cervical Cancer: Development of Targeted Therapies Beyond Molecular Pathogenesis" by Knoff et al., 2014, *Current Obstetrics and Gynecology Reports*, 3(1), 18–32. Copyright 2013 by Springer Nature.

Table 1

Summary of All Studies

Author (Year)	Title	Vaccine Type	Purpose	Methods	Endpoints	Results	Conclusions
Maciag (2009)	The first clinical use of a live-attenuated Listeria monocytogenes vaccine: A Phase I safety study of Lm-LLO-E7 in patients with advanced carcinoma of the cervix	Vector-based (Listeria)	To evaluate the safety of Lm-LLO-E7 in patients with advanced carcinoma of the cervix.	Phase I safety study, n=15, 3 dose levels as iv with 2nd dose 3 weeks later	Primary endpoint: Adverse events Exploratory: efficacy (tumour size regression and survival)	Mild to moderate AEs seen in all patients, no severe AEs related to the vaccine. 7.7% of patients had a reduction, 53.8% had a stable disease and 38.5% had a progressive disease.	These results show that cancer immunotherapy based on live-attenuated Lm, and possibly other bacterial vectors as well, is feasible and can become an alternative treatment in the future.
Hillemanns (2022)	A Therapeutic Antigen-Presenting Cell-Targeting DNA Vaccine VB10.16 in HPV16-Positive High-Grade Cervical Intraepithelial Neoplasia: Results from a Phase I/IIa Trial	DNA-based vaccine	To evaluate the safety, immunogenicity and efficacy of a therapeutic DNA vaccine VB10.16, using a unique modular vaccine technology that is based on linking antigens to CCL3L1 targetingmodule, in women with HPV16-positive high-grade cervical intrae-pithelial neoplasia (CIN).	Open-labe phase I/II CT, n=34, Two cohorts: two-dose and expansion	Primary end points: Adverse events Secondary endpoints: E6/E7 specific cellular response, HPV16 clearance, regression of CIN lesion size and grading	No serious adverse events, HPV-16 specifc T-cell responses in majority, HPV clearance = 47%, Reductions in lesion size = 94%, Regression (CIN 0/1) = 59%, significant correlation (P>0.001) between IFNγ T-cell response and lesion size reduction.	The novel therapeutic DNA vaccine VB10.16was well tolerated and showed promising evidence of efficacy andstrong HPV16-specific T-cell responses in subjects with high-grade CIN.
Komdeur (2021)	First-in-Human Phase I Clinical Trial of an SFV-Based RNA Replicon Cancer Vaccine against HPV-Induced Cancers	Vector-Based (Alphavirus-based theraputic vaccine (E6/7))	To assess immunological activity, safety, and tolerability of Vvax001, an aplhavirus based theraputic cancer vaccine against human papillomavirus (HPV)-induced cancers.	n=12, 3 cohorts of 4 by dose level (infectious particles per immuninzation), 3 immuniations with 3 week intervals	Adverse events, IFNγ response as T-cell response	Immunization with Vvax001 was safe and well tolerated, with only mild injection site reactions, and resulted in both CD4+ and CD8+ T cell responses against E6 and E7 antigens. Even the lowest dose of 5x10^5 infectious particles elicited E6/E7-specific interferon (IFN)-γ responses in all three participants in this cohort. Overall, immunization resulted in positive vaccine-induced immune responses in 12 of 12 participants in one or more assays performed.	Vvax001 was safe and induced immune responses in all participants. These data strongly support further clinical evaluation of Vvax001 as a therapeutic vaccine in patients with HPV-related malignancies.
Trimble (2015)	Safety, efficacy, and immunogenicity of VGX-3100, a therapeutic synthetic DNA vaccine targeting human papillomavirus 16 and 18 E6 and E7 proteins for cervical intraepithelial neoplasia 2/3: a randomised, double-blind, placebo-controlled phase 2b trial	Synthetic plasmids (E6/7)	Efficacy, safety, and immunogenicity of VGX-3100 were assessed in CIN2/3 associated with HPV-16 and HPV-18.	Randomised, double-blind, placebo-controlled phase 2b study, n=167, 6 mg vaccine or placebo at 0, 4, and 12 weeks (stratified for age and severity of CIN)	Primary endpoints: Efficacy: regression to CIN I or normal pathology 36 weeks after the first dose and safety: adverse events.	49.5% VGX-3100 recipients and 30.6% placebo recipients had histopathological regression (percentage point difference 19.0 [95% CI 1.4–36.6]; p=0.034). HPV clearance was seen in 40.2% of experimental participants and 14.3% of placebo participants. Injection-site reactions occurred in most patients, but erythema was significantly more common in the VGX-3100 group (78.4%) than in the placebo group (57.1%; percentage point difference 21.3 [95% CI 5.3–37.8]; p=0.007).	VGX-3100 is the first therapeutic vaccine to show efficacy against CIN2/3 associated with HPV-16 and HPV-18. VGX-3100 could present a non-surgical therapeutic option for CIN2/3, changing the treatment outlook for this common disease.
Kenter (2009)	Phase I Immunotherapeutic Trial with Long Peptides Spanning the E6 and E7 Sequences of High-Risk Human Papillomavirus 16 in End-Stage Cervical Cancer Patients Shows LowToxicity and Robust Immunogenicity	Long-peptide vaccine (E6 and E7) and montanide ISA-51 adjuvant	To determine the toxicity, safety, and immunogenicity of a (HPV16) E6 and E7 long peptide vaccine administered to end-stage cervical cancer patients.	Single-center, single-group, observationl phase 1 study, n=35, vaccination 4 times at 3-week intervals, Different dosing cohorts	Safety and toxicity, T-cell responses (IFNγ)	No toxicity beyond grade 2 was observed during and after four vaccinations. In a few patients, transient flu-like symptoms were observed. Enzyme-linked immunospot analysis of the vaccine-induced immune response revealed that coinjection of the E6 and E7 peptides resulted in a strong and broad T-cell response dominated by immunity against E6. Injection of the E6 and E7 peptides at two different sites increased the E7 response but did not affect the magnitude of the E6-induced immune response.	The HPV16 E6 and E7 long peptide-based vaccine is well tolerated and capable of inducing a broad IFNg-associated T-cell response even in end-stage cervical cancer patients.

Note. Table 1 summarises all study information from Hillemanns et al. (2022), Kenter et al. (2008), Komdeur et al. (2021), Maciag et al. (2009), and Trimble et al. (2015).

Table 2

Summary of Data from All Studies

Author (Year): Vaccine	Safety	IFNγ Response	HPV clearance	Lesion Regression
Maciag (2009	Mild to Severe Aes	N/A	N/A	7.7% Reduction 53.8% Stable 38.5% Progressive
Hillemanns (2022): VB10.16	Only mild AEs	Expansion cohort: 94% Intial dosing cohort 1: 84% Intial dosing cohort 2: 100%	Expansion cohort: 47% Intial dosing cohort 1 and 2: 38%	Expansion cohort: 71% - >50, 59% - to CIN 0 or I, 47% - Complete Intial dosing cohort 1: 75% - >50, 38% - to CIN 0 or I, 25% - Complete Intial dosing cohort 2: 50% - >50, 38% - to CIN 0 or I, 25% - Complete
Komdeur (2021): Vvax001	Only mild AEs	83% HPV-16 E6/E7	N/A	N/A
Trimble (2015): VGX-3100	Only mild AEs	N/A	Expermental: 40.2% Placebo: 14.3%	Expermental: 49.5% Placebo: 30.6%
Kenter (2009): Long-peptide vaccine	Only mild AEs	Cohort 1: 100% - E6 and 57.1% - E7 Cohort 2: 81.8% - E6 and 63.6% - E7 Cohort 3: 66.7% - E6 and 44.4% - E7	N/A	N/A

Note. Table 2 summarizes all study results from Hillemanns et al. (2022), Kenter et al. (2008), Komdeur et al. (2021), Maciag et al. (2009), and Trimble et al. (2015).