Vaccinations for Anal Squamous Cancer: Current and Emerging Therapies

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Abstract

Keywords

- ▶ anal cancer
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- ▶ anal squamous cell cancer

Human papillomavirus (HPV) infection is responsible for 4.3% of the global cancer burden. Since 2006, current HPV vaccines have reduced the prevalence of the virus in adolescent girls, reduced the prevalence of genital warts, and been proven to reduce the progression of anal intraepithelial neoplasia in men. Herein, we review the epidemiology, virology, and immunology behind the prophylactic HPV vaccines and current recommendations for its use. We also review future immune therapies being trialed for use against HPV-related cancers including anal cancer.

With current vaccines, all human papillomavirus (HPV)-related malignancies, including anal cancer, could be prevented with immunization before exposure to the virus. When considering a strategy of prophylactic vaccination, the question is how many people would have to be vaccinated to prevent an HPV-related cancer and at what cost. Herein we review the HPV, current recommendations for the HPV vaccine, and future directions of vaccine therapy for anal cancer.

Epidemiology

Roughly 79 million people in the United States are infected with HPV.¹ The prevalence of high-risk genital HPV, the most frequent and aggressive serotypes, in the U.S. population is estimated to be 22.7%.² Thus, 18 million people are infected with these highly oncogenic serotypes at any given time. This rate of infection includes a mix between those with new, persistent, and clearing infections.

In comparison to those infected, there were 39,000 HPV-related cancers per year in the United States between 2008 and 2012. For anal cancer, there is an average of 5,010 cases per year; 3,260 in women and 1,750 in men. The CDC estimates that 91% (4,600) of anal cancers were due to HPV infection. Of these, 79% were due to HPV 16 and 18, and 8% were probably caused by HPV 31, 33, 45, 51, and 58.

The incidence of infection is estimated to be increasing by 2% per year.³ The small percentage of those individuals who develop cancer versus the large number who are infected demonstrates that HPV infection does not always result in anal cancer.

Globally, HPV and HPV-related cancers are of a larger concern. According to GLOBOCAN 2012 database, approximately 4.3% or 600,000 of the 14.1 million cancers occurring worldwide annually can be attributed to HPV infections. Incidence varies substantially by region, with 1.7% of North American women suffering from HPV-related cancers compared with 25.2% in sub-Saharan Africa and 18.9% in South Central Asia.⁴ In general, the prevalence of HPV-related cancers is increased in geographic areas without formalized screening programs, places where a prophylactic vaccination is most needed.

Overall, HPV is responsible for approximately 5.2% of all human cancers.⁵ This is a tremendous disease burden, and while prophylactic vaccination against HPV can decrease this by improving global health, this strategy is not without challenges. Vaccines, like Gardasil and Cervarix, have been found to augment and boost the immune response of young women, but virus-like particle (VLP) technology is expensive, and the present vaccine distribution requires refrigeration. The realities of cost and scale make vaccine distribution in countries without screening programs, where it is most needed, difficult.⁶

HPV-related cancers have traditionally been studied in heterosexual men, women, men who have sex with men (MSM), and the immunosuppressed. As a whole, the incidence of HPV infection is slightly higher in women than in men.⁷ More men are infected in the 20- to 49-year-old range than women, but women are more likely to be infected in all other age groups.⁸

In HIV-negative women, the prevalence of anal HPV infection is between 27 and 42%. Most women will have an HPV infection at some point in their lives, as 70% of the women without anal HPV develop an infection during follow-up screening.⁹ In the United States, the prevalence of anal receptive intercourse in women is between 22 and 29%, slightly higher than in men, which may account for the higher incidence of anal HPV and cancer in women.¹⁰

The prevalence of anal HPV infection in heterosexual males is between 8 and 38%. A third of these infections are with high-risk HPV.¹¹ By definition, MSM make up a disproportional amount of men participating in anal intercourse, and the prevalence of anal HPV in MSM is greater than that in heterosexual males, at around 47%.¹² As a whole, immunosuppressed patients are more susceptible to HPV-related cancers with the highest risk of anal cancer being in MSM with HIV.¹³ Over 90% of HIV-infected MSM have a concomitant anal HPV infection, and invasive anal cancer is 37 times more likely in this group of men.¹⁴ Interestingly, the incidence of anal cancer among HIV-infected persons persisted despite treatment with antiretroviral therapy,¹⁵ but significantly less anal intraepithelial neoplasias (AINs) 2 and 3 were found in HIV-positive men who were on combination antiretroviral therapy for more than 24 months.¹⁶ In total, these data suggest that the incidence of HPV-induced neoplastic changes is more related to immune status, specifically CD4 status, and anal intercourse rather than gender alone.

Virology

The virus HPV is the near-perfect viral pathogen that does not significantly impair the host, is extremely infectious, and can avoid a significant immunologic response. The virology of HPV helps explain these characteristics and illuminates the mechanism of proposed treatments. HPV is a DNA virus that infects epithelium. There are over 100 known serotypes of HPV which infect humans. These serotypes are named by the order of their discovery.¹⁷ With regard to anal cancer, 15 of the 30 to 40 HPVs that infect the anal mucosa are carcinogenic.⁵ HPV has an 8-kb circular genome enclosed in a capsid shell composed of the L1 and L2 capsid proteins. These are the antigens present in today's HPV vaccines like the quadrivalent HPV vaccine (Gardasil) and the bivalent vaccine (Cervarix). The 8-kb genome encodes E1, E2, E4, E5, E6, and E7 proteins. These early genes are necessary for transcription. The E6 and E7 proteins are the immortalization proteins which manipulate cell cycle regulatory functions to block apoptosis and cell-mediated immune response causing neoplastic changes.¹⁸

Like many persistent and pervasive viruses, HPV survives by immune system evasion. Some of HPV's evasive mechanisms include the lack of a viremic phase, cytolysis, or inflam-

mation, and the infection of epithelium without activation of dendritic cells (DC). HPV inhibits interferon and receptor signaling, preventing a robust immune response. Additionally, viral particles are shed from the luminal surface, not the basement membrane. These luminal epithelial cells have little access to the vascular and lymphatic channels required for transport of immune cells to lymphoid organs.¹⁹ HPV's ability to go undetected by the human immune system has been in place since the origin of humanity, as the genome of the virus has changed only 2% since that time.²⁰ These long-established escape mechanisms have made HPV one of the most common sexually transmitted infections world-wide.²¹ Given the lack of symptoms in the host, there is a need for both screening and prophylactic vaccination in the prevention of neoplastic changes.

The Vaccine

Understanding the HPV, L1 VLP vaccines, and the immune response is crucial to improve efficiency of vaccination and potential immunization short-falls. The immunoadjuvant in Gardasil is amorphous aluminum hydroxyphosphate sulfate,²² while the Cervarix uses aluminum hydroxide and 3-desacyl-4'-monophosphoryl lipid A.²³ When given as prophylaxis, the vaccines are 100% effective against persistent cervical infection with HPV 16/18 by generation of antibodies.²⁴ This protection against high-risk HPV persists up to 4.5 years with 97% of patients protected against incident infection and 100% clearance of HPV infections at 12 months. This resulted in a near 100% efficacy against cervical intraepithelial neoplasia (CIN) associated with the vaccine serotypes.²⁵

In comparison, the natural humoral immune response to HPV infection is less robust. In women, 54 to 69% develop type-specific antibodies to oncogenic strains after infection, and in men, the type-specific antibody rates are even lower at 4 to 36%. Overall, in men and women, the development of type-specific antibodies to HPV16 occur 13% of the time. Interestingly, HPV is often cleared in the immunocompetent host despite the lack of antibody response. This is in sharp contrast to vaccination with Gardasil or Cervarix where 100% of those inoculated develop antibodies.^{26,27} Thus, the CD8 cytotoxic lymphocyte and CD4 helper T cell likely play a larger role in the elimination of infection. Despite the lack of antibodies, half of the infections in immunocompetent women will be cleared in 6 months, and 90% will be cleared within a few years after acquisition.²⁸ Once the infection is cleared, immunologic protection persists. In the FUTURE II study group and the PATRICIA trial, less than 1% of those women who were HPV seropositive and DNA negative developed HPV 16/18 infections, while HPV seronegative women developed infections at twice the rate.^{29,30} Other studies confirm that despite reinfection, intraepithelial neoplasia does not occur.³¹ In men, Nyitray et al showed a persistent infection with HPV 16 in 5.1% in MSM but a complete absence in heterosexual men.³² With such effective clearance and lasting immunity against reinfection, it is difficult to understand the role of a vaccine in the prevention of HPV infection and associated cancers in the immunocompetent host.

Natural Course of Disease from AIN to Anal Cancer

Data suggest AIN's progression to anal cancer is somewhat variable. Small prospective studies in HIV-negative, immunocompetent patients determined the risk of AIN 2 or 3 progressing to anal cancer is between 9 and 13% and 0% for AIN 1.³³ However, in a meta-analysis performed by Machalek et al, the progression from AIN 2 or 3 to anal cancer is much lower even in high-risk populations: 1 in 4,000 for HIV-negative MSM and 1 in 600 for HIV-positive MSM.³⁴ Critical analysis of the double-blind, randomized controlled trial by Palefsky et al that examine the subset of MSMs. In 602 men followed for 36 months, not one developed anal cancer, although 5 cases of AIN 2 or 3 were observed in the arm receiving prophylactic VLP-1 vaccination and 24 in the placebo arm.³⁵ The theory behind this phenomenon can only be seen as either low rates of progression or high rates of regression. Either way, this raises the question of which treatment strategy is more efficient: screening with treatment or prophylactic vaccination.

Treatments of Anal Cancer and Their Effectiveness

The typical treatment for anal cancer is chemoradiation.³⁶ Norman Nigro introduced his chemoradiation protocol in 1974.³⁷ His concurrent use of radiation therapy with Mitomycin and 5-fluorouracil yielded 5-year survival rates of ~80% in 1996.³⁸ Now, the overall 5-year survival rate for those diagnosed with anal cancer is 66%. The 5-year disease-free survival (DFS) for Stage I disease is 80%. With regional spread, the 5-year DFS is 60%, and it drops to 31% with metastatic disease. In 2016, 0.0024% of the U.S. population is predicted to be diagnosed with anal cancer, 8,080 of 324.1 million people. Thirteen percent of the 0.0024%, or 0.00003%, will die from the disease.³⁹ If other benign treatment modalities, such as screening and treatment, were as effective as prophylactic vaccination, then millions of dollars could be saved by eliminating unnecessary vaccinations.

Current Recommendations

Given both the low incidence and overall mortality of anal cancer, HPV vaccination for reducing anal cancer alone offers only small gains in the general population. However, the benefit for HPV-related cancers is five times that for just anal cancer alone. The HPV vaccine is also working to reduce the prevalence of the virus, as well as that of genital warts, in adolescent girls.^{40,41} Thus, recommendations for vaccination have evolved and broadened. Since Gardasil was licensed in 2006, current recommendations of the Advisory Committee on Immunization Practices (ACIP) by the CDC are as follows:

1. Routine vaccination at the age of 11 or 12 years with Gardasil or Cervarix for females and with Gardasil only for males. The vaccination is safe to administer at 9 years of age. Both vaccines should be administered in three doses.

The second vaccine should be given 1 to 2 months after the first, and the third inoculum should be given 6 months after the first dose.⁴²

2. Data support the vaccination of women aged 13 to 26 and men aged 13 to 21 years if those individuals were not vaccinated prior or did not complete the three-dose series.
3. Men aged 22 to 26 may be vaccinated.
4. If males or females reach age 27 prior to completing the three-dose series, the remaining doses can be administered to complete the vaccination series.
5. HPV vaccination is recommended in those with known HPV infection, those who are immunocompromised, and in MSM up to age 27.⁴³

In 2015, the ACIP approved the 9-valent HPV vaccine for use in routine vaccination in individuals up to 27 years of age. The 9-valent HPV vaccine, Gardasil 9, is effective against HPV 6, 11, 16, and 18 like Gardasil, and also covers serotypes 31, 33, 45, 52, and 58. The five additional types are mostly involved with cervical cancer; so, the 9-valent vaccine provides females with more protection.⁴⁴ Starting in 2017, only the 9-valent HPV vaccine is available in the United States. In an effort to increase vaccination, the CDC recommended only two inoculations, 6 months apart for 11- and 12-year-olds in October 2016. Older teens and adults should continue a three-dose series.⁴⁵

HPV-Specific Immunology

Understanding HPV and cell-mediated immunity is crucial to understanding future therapies. HPV's life cycle is exclusively intracellular. Thus, a humoral response or antibody producing vaccinations cannot interrupt that life cycle once the infection is established. Instead a cell-mediated, T-cell response is required to recognize and kill host cells infected with the virus and thus prevent virus-induced neoplasias. T-cells are activated by antigen presenting cells and in the case of the epithelial-based HPV antigen, Langerhans cells (LCs). As a DC, the LC is the key to the cellular immune response. LC cytokine release helps determine the mixture of T-cells and the differentiation of CD4 helper T-cells. The three T-cell populations integral to a complete, sustained, and effective cell-mediated immune response are CD8 cytotoxic T-lymphocytes, CD4 cells, and regulatory T-cells or T-regs. CD8 cells recognize intracellular peptides displayed on MHC, class 1 molecules. CD4 cells secrete cytokines to upregulate CD8. The pattern of cytokine secretion determines CD4 subclassifications into Th1 or Th2 cells. Finally, T-regs regulate responses to both self and exogenous antigens.

As mentioned earlier, HPV evades the innate and gently activates the adaptive immune system. The viruses suppress a cell-mediated, antiviral response by decreasing cellular type 1 interferon production in response to infection. The virus does this in concert with the other natural immune evasive mechanisms described earlier in the chapter. The prolonged, low-level exposure to HPV antigen induces tolerance. Despite this *in vivo* tolerance, Alexander et al demonstrated that *in vitro* activated, CD8, cytotoxic T-lymphocytes

(CTL) can lyse HPV-16 infected cells.⁴⁶ Additionally, immunohistochemical analysis of cervical cancer specimens demonstrates the presence of activated CTLs within lesions.⁴⁷ In addition, HPV-16, E6 CTLs have been identified in the lymph nodes of cervical cancer patients.⁴⁸ The presence of CTL within lesions and in regional lymph nodes demonstrates that the cell-mediated suppression by HPV is not complete. Thus, cell-mediated effectors are capable and present, but the scale of the immune response is inadequate.

The deficiency of scale is best illustrated by comparing women who clear or do not clear the HPV16 infection. Effector CTL phenotypes are more prevalent in women with HPV16 infection who have not developed intraepithelial changes than in those who have. This lack of CTL response to the HPV-16, E6 protein has been correlated with persistent infection.⁴⁹ A robust, sustained CTL or CD8 T-cell response is necessary for clearance of the HPV infection; without it, infection persists and epithelial changes occur. The reason for the lack of CTL response is likely due to downregulation of

MHC1 expression and interferon secretion induced by the virus' E6 and E7 oncoproteins.⁵⁰

Future Directions

Overcoming the mature, evolved, immune evasion and survivability traits of the HPV has proven to be difficult, but many immunotherapy trials are underway. Before reviewing these trials, it is important to understand the spectrum of vaccine modalities. These modalities are best understood by reviewing antigen presentation and CTL priming (see ▶Fig. 1). Below, we outline a few clinical trials and review their proposed mechanisms in the context of anal cancer and other HPV-related malignancies. In this section, more attention is given to newer and more effective therapies like check point inhibitors (CPIs) as well as viral vector and peptide vaccines.

CPIs are a newer class of antitumor drugs that inhibit regulatory T-cell inhibition of CTLs. There are two major targets for CPIs, CTLA-4 on regulatory T-cells and CTLs and

Fig. 1 (a) Viral vector, DNA, tumor lysate, autologous tumor cells, peptide vaccines, and viral-like particles deliver tumor-associated or viral antigens to dendritic cells (DCs). (b) These DCs activate and travel to lymphoid centers where they in turn activate T-cells and B-cells in a response to the tumor-associated antigen (TAA). A different set of cancer vaccines, called DC vaccines, utilize activated, autologous DCs, or tumor lysate, particle-loaded DCs. Prepared ex vivo and infused, these DCs then activate T- and B-cells. (c) Activated T-cells or cytotoxic T-lymphocytes (CTLs) can also be utilized in vaccines. In preparation for vaccination, CTLs undergo extraction from the patient, priming against TAAs, and activation with cytokines ex vivo prior to infusion. These effector cells then migrate to tumor sites eliminating infected host cells. (d) T-cells, of all types, migrate to the site of neoplastic cells and are then subject to the tumor microenvironment. Here, CTLs or tumor infiltrating lymphocytes are acted upon host, neoplastic cells and regulatory T-cells. These signaling pathways are the target of check point inhibitors like Nivolumab (program cell death receptor-1 [PD-1] inhibitor) and Ipilimumab (cytotoxic T-lymphocyte-associated molecule 4 [CTLA-4] inhibitor). PD-1 receptors are expressed on activated T-cells and other myeloid cells. Blocking of the interaction between PD-1 ligand and PD-1 allows CTLs to avoid inhibition, continuing IL-2 production and proliferation. In a separate pathway, activation of the CTLA-4 receptor, present on regulatory and CTLs, CD80 on DCs, inhibits CTL proliferation. Ipilimumab blocks the CTLA-4 from being activated, preventing subsequent inhibition of an immune response.

PD-1 on CTLs alone. New targets for CPIs are developed weekly. The inhibitory interaction between CTLA-4 (CD152) on regulatory T-cells with B7 and on CTLs prevents CTL activation. CTLA-4 inhibitors, like Ipilimumab, prevent the inhibition, allowing tumor infiltrating CTLs to recognize and lyse cancer cells. This results in a new antigen cascade for DCs and a robust cell-mediated response. Activation of the PD-1 ligand on the surface of CTLs promotes apoptosis. PD-1 inhibitors like Nivolumab and Pembrolizumab prevent CTL apoptosis, allowing tumor cell lysis and a robust immune response. Since CTLs have been seen in histologic samples of human HPV-related cancers⁵¹ and a functional depletion of regulatory T-cells has been shown to induce a strong intratumoral invasion of CTLs with eradication of HPV tumors in 70% of animals, there exists a proven mechanism for clinical trials to progress.⁵² A phase 2 clinical trial of single agent, systemic Nivolumab in refractory metastatic anal canal cancer (NCT02314169) shows a disease control rate in 70% of patients. In the next step beyond systemic administration, clinical trials in HPV-related head and neck cancer have begun intratumoral injection of Ipilimumab prior to surgery.⁵³ With proof of concept, intralesional injection of CPIs should begin to be introduced in the treatment of premalignant, anal lesions: a space in which viral vector vaccines have already found success.

Viral vector vaccines consist of an attenuated virus transduced with genes encoding tumor-associated antigens (TAAs) and immunoadjuvants. TAAs are antigenic peptide and partial peptide sequences specific to cancer cells and absent in normal cells. These attenuated, immunogenic viruses express the selected TAAs, and the immune response to the virus induces a T-cell response to the TAAs. One such vaccine is the recombinant, modified Vaccinia Ankara (MVA) virus designed to express the HPV E2 protein. The MVA E2 vaccine has been used to treat high-grade cervical intraepithelial lesions via intracervical injection. Using this local injection strategy, 59% of those treated showed no lesions on repeat colposcopy, while 33% had reduction in the size of the CIN, 3 weeks after the vaccine series.⁵⁴ The success of this phase 2 trial leads to a large phase 3 trial in mostly cervical lesions and also included anal intraepithelial lesions. Vaccination with the MVA E2 resulted in complete elimination by histology in 89% of women and 100% of men. In fact, 83% eliminated HPV DNA completely.⁵⁵ This viral vector vaccine represents a major step forward and one that is more economically feasible than a prophylactic strategy in countries with screening programs. Certainly, this vaccine is worth further investigation in the United States.

Peptide vaccines are based on inoculation of a patient with immunogenic epitopes derived from a TAA. These peptides are processed in vivo and presented to T-cells. This strategy allows investigators to choose one or more specific antigens to prime T-cells against. Peptide TAAs can be easily mass produced but rely on endogenous processes for presentation of TAAs. The success of peptide vaccines is limited. One hypothesis for the limited success is the induction of regulatory T-cells along with CTLs. In a phase 2 trial for high-grade vulvar intraepithelial neoplasia, 19 women were treated with Imiquimod for 8 weeks followed by a

primary vaccination series with a HPV16 E6, E7, and L2 fusion protein. Sixty-three percent showed regression at 1 year. There was significant CD8 and CD4 infiltration on histology in those who responded, and nonresponders showed an increase of regulatory T-cells. These are modest results, but a closer look can highlight the weaknesses of a peptide vaccine and the opportunity to combine CPIs with peptide vaccines. Both CTL and regulatory T-cell populations are stimulated in response to peptide antigens, but with CPIs, the opportunity to limit the impact of regulatory T-cells now exists. A combined, synergistic multi-immunotherapy treatment is being proposed for other malignancies^{56,57} and should be further explored in anal cancer.

In anal cancer, trimodality therapy has improved survival over surgery alone, but only minor improvements have been achieved in the past 40 years. A prophylactic vaccination strategy with a multivalent VLP vaccine is cost-effective and prevents anal cancer precursor lesions. However, the prophylactic vaccines cannot be used to treat anal precursor lesions once the virus is contracted. The efficiency of a prophylactic strategy must soon be compared with new, viable vaccine therapies used in a screen and treat strategy. When these vaccines undergo approval, instead of vaccinating millions of unaffected people, only those patients with precursor lesions would undergo inoculation in such a strategy. Thus, in countries with organized screening, therapeutic vaccines administered to prevent progression of identified AIN to cancer will be a feasible and a more efficient option. Worldwide, combining these two immunologic strategies will offer the best outcomes. The need for a prophylactic strategy would exist in underdeveloped countries without screening, unlike the United States and Canada.

Vaccines and immunotherapy are way ahead for many malignancies, and anal cancer is no exception. Future trials for anal cancer should focus on a synergistic approach: vaccination with TILs, DCs, ATCs, TAAs, or viral vector vaccines followed by intratumoral injection of CPIs. The next step in preventing anal cancer and improving survival in local regional disease will be an immunotherapy.

Disclosures

The view(s) expressed herein are those of the author(s) and do not reflect the official policy or position of the U.S. Army Medical Department, the U.S. Army Office of the Surgeon General, the Department of the Army, Department of Defense, or the U.S. Government.

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