Human Immunodeficiency Virus Infection Promotes Human Papillomavirus-Mediated Anal Squamous Carcinogenesis: An Immunologic and Pathobiologic Review

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Keywords

Anal cancer · Squamous cell carcinoma · Human immunodeficiency virus · Human papillomavirus

Abstract

Background: Anal squamous cell carcinoma (SCC) is a rare gastrointestinal malignancy with rising incidence, both in the United States and internationally. The primary risk factor for anal SCC is human papillomavirus (HPV) infection. However, there is a growing burden of disease in patients with human immunodeficiency virus (HIV) and HPV coinfection, with the incidence of anal SCC significantly increasing in this population. This is particularly true in HIV-infected men. The epidemiologic correlation between HIV-HPV coinfection and anal SCC is established; however, the immunologic mechanisms underlying this relationship are not well under-

stood. **Summary:** HIV-related immunosuppression due to low circulating CD4⁺ T cells is one component of increased risk, but other mechanisms, such as the effect of HIV on CD8⁺ T lymphocyte tumor infiltration and the PD-1/PD-L1 axis in antitumor and antiviral response, is emerging as significant contributors. The goal of this article is to review existing research on HIV-HPV coinfection anal SCC and precancerous lesions, propose explanations for the detrimental synergy of HIV and HPV on the pathogenesis and immunologic response to HPV-associated cancers, and discuss implications for future treatments and immunotherapies in HIV-positive patients with HPV-mediated anal SCC. **Key Messages:** The incidence of anal squamous cell carcinoma is increased in human immunodeficiency virus (HIV)-infected patients, even in patients on highly active antiretroviral therapy. Locoregional HIV infection may enhance human papillomavirus oncogenicity. Chronic inflammation due to HIV infection may contribute to CD8⁺ T lymphocyte exhaustion by upregulating PD-1 expression, thereby blunting cytotoxic antitumor response.

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Introduction

Anal squamous cell carcinoma (SCC) is a rare gastrointestinal (GI) malignancy that arises within the mucosa of the anal canal [1, 2]. Although only comprising 3–4% of all GI malignancies, its incidence is growing; in 2004 and 2020, 3,500 and 8,500 new cases were diagnosed in the United States, respectively [1, 3–5]. In 2021, it is estimated over 9,000 patients will be diagnosed, and 1,400 patients will die from anal SCC [6–8]. Over 60% of patients with anal SCC are women; however, a growing proportion of men are developing the disease, particularly men who have sex with men (MSM) [1, 6, 7, 9]. While screening programs exist in some areas of the United States, they are not standardized or widespread; increased awareness will be crucial for improved prevention and patient outcomes [10].

Although classified as a GI malignancy, anal SCC is similar to other genital squamous cancers in etiology, risk factors, and affected patient population. The anal canal is lined with squamous epithelium, with the transition to columnar rectal mucosa centered at the dentate line. The anal canal mirrors the milieu of the cervix, which is reflected in the distribution of cancer types arising there [1]. Risk factors include smoking, receptive anal intercourse in men, genital human papillomavirus (HPV) infection, and, increasingly, human immunodeficiency virus (HIV) infection [3–7, 9, 11]. The first-line treatment for anal SCC is chemoradiotherapy, with some cases requiring surgery. Five-year survival estimates are 70–80%, although significant morbidity is associated with post-surgical wound complications. In the 10–20% of patients who present with metastatic disease, 5-year survival decreases to below 20%, emphasizing the importance of early diagnosis and treatment [1, 6, 7, 9, 12].

HPV-Mediated Anal Squamous Cell Carcinogenesis

HPV is a double-stranded DNA virus with over 200 known strains and is the most common sexually transmitted infection worldwide [13–15]. In anal infections, “low risk” strains, including types 6 and 11, cause benign squamous papillomas or condyloma acuminata; these have a very low risk of progression to invasive SCC, with a slightly higher risk in immunocompromised patients [16, 17]. In contrast, “high risk” strains (hrHPV), including types 16 and 18, may induce squamous intraepithelial lesion formation, which can ultimately progress to anal SCC [18, 19].

Anal SCC is highly associated with HPV infection, and hrHPV infections are responsible for the vast majority of precancerous lesions. Types 16 and 18 are responsible for over 80% of HPV-associated anal SCC. These 2 strains are also responsible for over 90% of HPV-associated cervical and oropharyngeal SCC [20–23]. The role of HPV in the pathogenesis of anal squamous intraepithelial lesions and resultant SCC is well-described [24–27]. Cells found within transitional squamocolumnar zones are thought to be more vulnerable to HPV infection and resultant dysplasia [28, 29]. hrHPV strains infect epithelial cells chronically, and high viral burden and long duration of infection increase the likelihood of precancerous lesion formation [30].

HPV initially infects basal cells of the anal canal epithelium using viral late proteins L1 and L2. Once initial infection occurs, early viral genes E1–E8 facilitate viral replication and viral protein translation. Of particular note, oncoproteins E6 and E7 interact with and cause degradation of cell cycle regulators p53 and Rb in epithelial cells, respectively (Shown in Fig. 1). Reduced activity of p53 and Rb results in viral genome replication, chromosomal instability, and epithelial cell proliferation, resulting in dysplasia and eventual progression to malignancy [31–36].

HIV-HPV Coinfection in Patients with Anal Squamous Intraepithelial Lesions and Anal SCC

Patients with HIV-HPV coinfection are increasingly recognized as having an elevated risk of anal precancerous lesions and subsequent anal SCC [3, 4, 6, 7, 9, 37–41]. Indeed, anal SCC is one of the most common cancers in HIV-infected patients, even in those with normal CD4+ cell counts [37, 42–46]. The risk of anal SCC is 120-fold higher in HIV-infected individuals than in noninfected individuals [47]. In addition, HIV-HPV coinfection has been increasingly implicated in cervical, penile, oropharyngeal, vulvar, and conjunctival SCC [48–50]. The proportion of anal SCC associated with HIV-HPV coinfection is higher in men, as over 25% of men with anal SCC are HIV-positive as opposed to approximately 1% of women [37, 51, 52]. Further, HIV-infected MSM have a 37-fold increased risk of developing invasive anal SCC compared to their HIV-negative counterparts [19].

The increased risk of anal SCC in HIV-HPV coinfecting individuals is likely multifactorial in etiology. First, as patients with HIV have gained access to improved medical management and longer life spans, the incidence of HIV-associated malignancies has increased [38, 42–46].

Fig. 1. Following HPV infection of anal squamous epithelium, HPV DNA is integrated into the human host cell genome. Expression of integrated DNA results in creation of HPV oncoproteins E6 and E7, which are crucial for HPV-mediated oncogenesis. E7 inhibits the tumor-suppressing protein Rb, deregulating cellular proliferation;

E6 blocks apoptosis of infected cells by promoting p53 degradation. The combined activities of E6 and E7 enhance proliferation of deregulated infected squamous cells, resulting in squamous intraepithelial neoplasia, and facilitating eventual progression to SCC. SCC, squamous cell carcinoma; HPV, human papillomavirus.

Furthermore, studies have found higher frequencies of coinfection with multiple strains of HPV in HIV-infected patients at oral and anogenital sites, compared to those without HIV [53–56]. As some lineages and sub-lineages of HPV display biological characteristics associated with persistent infections and evolution to cancer development, patients with HIV with a high HPV strain diversity may increase the risk of carcinogenesis [53–56].

Another proposed explanation for the increased risk of anal SCC in HIV-infected patients is HIV-related immunosuppression, as less potent immune responses impair the clearance of HPV-infected cells. Importantly, diminished cell-mediated immunity in the blood results in decreased immune function and increased the risk of anal SCC and precursor lesions [37, 57–62]. Lower pre-highly active antiretroviral therapy (HAART) CD4⁺ T-cell counts have been linked to higher recurrence risk in HIV-infected patients with HPV-associated anal SCC [63, 64]. Further evidence supporting immunosuppression as a risk factor for HPV-associated anal SCC is seen in patients receiving immunosuppressive medications following solid organ transplant. In this population, the development of anal precancerous lesions occurs significantly more

frequently compared with immunocompetent patients [65–67].

However, immunosuppression indicated by low CD4⁺ T-cell count is unlikely to fully explain the increased rates of anal squamous intraepithelial lesions and subsequent SCC in HIV-infected patients. Crucially, the incidence of anal SCC has continued to increase despite the widespread prescription of HAART medications [68–71]. Even in patients with normal CD4⁺ T-cell counts, for example, the risk of anal SCC remains elevated compared with HIV-noninfected patients [72–75]. Most likely, other HIV-related immunomodulatory mechanisms are at play. Notably, it is postulated that synergistic HIV-HPV interactions in the anal squamous microenvironment confer higher oncogenic risk [76].

The Role of HIV Infection in Enhancing the Oncogenic Potential of HPV

The mechanism of synergy between HIV and HPV is controversial and not completely understood. Along with population-level evidence that HIV-HPV coinfection

occurs in the same individuals, there is evidence that coinfection occurs locoregionally in the anal epithelium within an individual, albeit not within the same cells. First, HPV infection is initiated in basal epithelial cells, and it is hypothesized that HIV infection may elicit changes in the epithelial integrity, increasing the likelihood of acquiring HPV coinfection [77].

Furthermore, it is thought that this coinfection bidirectionally influences the pathophysiology of each virus. Although HIV was initially thought to primarily enter cells via CD4 surface receptors, recent research suggests that HIV binds additional receptors, including CXCR4 and CCR5 [78]. CXCR4, along with its ligand CXCL12, is a highly conserved cell leukocyte surface marker involved in cell migration and chemotaxis across many species [79]. Similarly, CCR5 is primarily expressed on leukocytes and participates in several immune modulation pathways [78, 80]. Both receptors facilitate HIV entry into cells by associating with the HIV envelope glycoprotein, allowing membrane fusion and viral internalization. Following entry into cells, HIV viral gene expression and replication occurs, notably as a result of HIV Tat protein activity [81]. Immune cells co-expressing CD4, CXCR4, and CCR5 are more abundant in the distal GI tract [82, 83]. As such, epithelial cells in this area are not only susceptible to HPV-associated anal lesions but also exposed to abundant infected immune cells and thus locoregional HIV infection. Furthermore, existing HPV-associated anal lesions have been shown to have a higher abundance of CD4+ T cells, dendritic cells, and macrophages, which are all known targets for HIV infection, thus potentially facilitating later acquisition of HIV infection in the anal canal [84, 85].

Regardless of the order in which a patient is infected, coinfection with HPV and HIV likely increases the oncogenicity of hrHPV. Previous studies suggest that this increased oncogenic risk is indirect, resulting from HIV-induced changes to the immune system and lower immune response to HPV [86]. However, more recent research suggests that HIV coinfection changes the shared microenvironment in the anal squamous mucosa to enhance HPV-associated oncogenesis [87, 88]. One possible explanation centers on the role of HIV Tat protein in magnifying HPV oncogenicity.

As described above, the HIV Tat protein is integral to viral genome replication. After HIV infects cells, HIV RNA is reverse transcribed into DNA prior to the production of viral proteins. This DNA is then integrated into the host cell genome through the activity of viral integrase and transcribed by the host's RNA polymerase II. HIV

Tat binds RNA polymerase II as well as other proteins required for transcription, thus promoting viral DNA transcription and viral replication [81, 87]. Evidence suggests that the presence of HIV Tat not only enhances HIV gene expression but may also increase gene expression of other viral DNA in cells with productive intracellular levels of HIV Tat [81, 87–89].

This role of HIV Tat in viral-associated oncogenesis has been previously demonstrated in Kaposi sarcoma: Tat stabilizes the oncogenic protein Kaposin A and promotes viral genome replication [90, 91]. A similar role for HIV Tat within HPV-associated oncogenesis has been previously proposed; Tat may increase the expression of oncoproteins E6 and E7, as well as protein E2, which is responsible for HPV genome replication [92, 93]. This interaction has been demonstrated in HPV-associated cervical and oral SCC, but not yet in anal SCC. In one study, Tat not only upregulated HPV-associated oncogenesis, but also reduced p53 protein levels thus increasing malignant potential [94–96]. Although HIV does not directly infect cells of the anal epithelium, local HIV-infected immune cells may generate productive levels of the Tat protein. Tat may then be secreted by HIV-infected immune cells and taken up by local cells [88, 89] (shown in Fig. 2). The potential entrance of HIV Tat into anal epithelial cells may increase HPV gene expression and thus facilitate precancerous lesion formation and progression to anal SCC.

The Effect of HIV Coinfection on Antitumor Response

CD8+ T-Cell Infiltration

HIV coinfection likely modulates the cell-mediated immunologic response to anal precancerous lesions beyond initial vulnerability to HPV infection and direct impairment and depletion of CD4+ T cells. Specifically, HIV may alter CD8+ T lymphocyte function, which is central to the immunologic response in HPV-associated lesions. This has been shown in the setting of HPV-associated cutaneous warts, in which cell-mediated immunity is a significant predictor of clearance [97]. Similarly, in hrHPV infection, the level of CD8+ T-cell-mediated immune response is a significant predictor of lesion progression and future oncologic risk [98–102]. HPV-infected cells are particularly susceptible to CD8+ T-cell-mediated attack, as oncoproteins E6 and E7 are processed and presented by human leukocyte antigen class I. E6- or E7-specific CD8+ T cells can therefore induce apoptosis of HPV-infected cells, mainly through secretion of perforin and granzyme B [85].

Fig. 2. As HIV replicates within infected immune cells, the HIV Tat protein is produced and enhances HIV viral genome replication and HIV DNA transcription. HIV Tat is then secreted by infected immune cells and taken up by HPV-infected anal epithelial cells. Here, HPV DNA is integrated into the host cell genome, and HIV Tat facilitates increased transcription of HPV oncoproteins E6 and E7. HPV, human papillomavirus; HIV, human immunodeficiency virus.

The antitumor effect of CD8⁺ T cells directed toward tumor-associated antigens is similar to that of CD8⁺ T cells directed against HPV-infected cells described above [103–106]. In anal SCC, increased density of CD8⁺ T lymphocyte infiltration correlates with improved local control, treatment response, and survival outcomes [107–109]. However, one study showed the opposite trend in cells that were positive for CD8 and granzyme B [110]. This finding suggests that not only the presence of CD8⁺ T cells, but also their phenotype and activity, are likely important as well.

The cell-mediated immune response has also been linked to improved outcomes in other HPV-related cancers. In patients with hrHPV-associated oropharyngeal SCC, increased CD8⁺ T-cell infiltration predicts improved survival [111–113]. A similar association exists in cervical cancer, with lymphocytic infiltration being associated with a lower likelihood of metastasis and improved prognosis [114, 115].

PD-1/PD-L1 Axis

Another mechanism implicated in the immunologic response to anal SCC is the programmed death 1 (PD-1)/PD-ligand 1 (PD-L1) axis. PD-1 is an inhibitory receptor expressed on T cells, and it controls T-cell function and proliferation through interacting with PD-L1, which can be expressed by regulatory T cells, myeloid cells, and tumor cells themselves. Ongoing inflammation and active immune response, particularly secretion of interferon-gamma, cause increased PD-L1 expression and thus activate the PD-1/PD-L1 axis. Physiologically, this serves as an important check on a potentially overactive immune response and prevents immunopathology [116].

Although central to appropriate T-cell development and physiology, the PD-1/PD-L1 axis is often harnessed by tumor cells to evade the immune response. Tumor cells that express PD-L1 activate PD-1 on T cells, thereby inhibiting a local immune response and escaping cytotoxicity. As such, PD-L1 expression on tumor cells has been

shown to be an indication of worse prognosis in patients with solid tumors [117]. The PD-1/PD-L1 axis has also been implicated in HPV. Oncoproteins E6 and E7 increase PD-L1 expression, thereby activating the axis and decreasing local immune response to HPV-infected cells [118].

The current literature is mixed regarding the significance of the PD-1/PD-L1 axis in HPV-associated anal SCC. PD-L1 expression in anal SCC has been linked with increased disease recurrence and decreased survival [119, 120]. Similarly, PD-L1 expression has been linked to the progression of precancerous lesions [121]. However, other studies have shown that PD-L1 expression indicates improved overall survival. As mentioned above, CD8+ T-cell-derived interferon-gamma cytokine can increase PD-L1 expression on tumor and myeloid cells in the tumor microenvironment. With this in mind, both the extent of CD8+ T-cell infiltration and PD-L1 expression should be taken into consideration when evaluating the significance of this axis in patient survival [109, 122].

CD8-PD-L1 Interplay

The interaction between infiltrating CD8+ T lymphocytes and the PD-1/PD-L1 axis has also been identified in the immunologic context in which anal SCC and anal precancerous lesions form. Active CD8+ T lymphocytes express PD-1, which physiologically serves as a balancing mechanism to inhibit active immune responses [123]. In the context of CD8+ T-cell-directed antitumor responses as described above, tumors expressing PD-L1 may evade CD8+ T-cell cytotoxicity [124].

The relationship between CD8+ T-cell infiltration and the PD-1/PD-L1 axis is equally complex in other cancers. In HPV-associated penile SCC, PD-L1 expression on tumor cells was linked to lower CD8+ T-cell infiltration and poorer survival outcomes [125]. However, in HPV-associated oropharyngeal SCC, both PD-L1 expression and CD8+ T-cell infiltration were associated with improved survival, although their relationships to one other were not explored [126]. In non-HPV-related cancers, the literature is also mixed. One study showed that PD-L1 expression on tumor cells was associated with lower CD8+ T cell infiltration and poorer outcomes [127]. However, in colorectal cancer and hepatocellular carcinoma, PD-L1 expression was associated with increased CD8+ T-cell infiltration. Both studies explain PD-L1 expression as a result of long-standing CD8+ T-cell infiltration [128, 129]. However, a study on hepatocellular carcinoma showed that CD8+ T-cell infiltration was a positive prognostic

indicator in low PD-L1-expressing tumors, and a nonsignificant marker in high PD-L1-expressing tumors [129]. This finding highlights the need for delineation of the temporal expression of PD-L1 so that primary tumoral expression can be differentiated from PD-L1 expression as an adaptive immune escape response.

HIV-Mediated Modification of Anti-tumor Immune Responses

HIV-HPV coinfection further modifies the immunologic context in which anal SCC forms, and how it impacts the immunologic response to tumor cells. One significant way in which HIV decreases the response to HPV-associated anal precancerous lesions, and SCC is via decreased circulating CD4+ T lymphocytes in patients whose HIV load is uncontrolled. CD4+ T cells play a critical role in sustaining CD8+ T-cell-mediated immune response [130]. As such, uncontrolled HIV infection has been shown to reduce CD8+ T lymphocyte antitumor activity, subsequently facilitating the progression of precancerous lesions into malignancy.

HIV has also been shown to directly decrease CD8+ T lymphocyte activity and efficacy. As advancements in medical management of HIV have significantly reduced the likelihood of HIV progression to AIDS (defined by CD4+ T cell count <200 cells/mm³), HIV is increasingly viewed as a chronic disease that can be closely managed. However, despite careful management, HIV infection induces a state of chronic inflammation throughout the body associated with increased inflammatory markers and chronic immune activation, and, notably, chronic activation of CD8+ T cells [131]. This state of chronic immune activation leads to CD8+ T lymphocyte exhaustion [132–134]. CD8+ T-cell exhaustion is thought to be mediated in part by the modulatory effects of HIV on PD-1/PD-L1 expression; chronic activation of CD8+ T lymphocytes leads to increased PD-1 expression [135–138] (shown in Fig. 3). Scientific understanding of the significance of the PD-1/PD-L1 axis in chronic HIV infection is evolving, with increasing evidence that PD-L1 expression is induced on a variety of cell types by chronic HIV infection. As discussed above, PD-L1 expression on tumor cells is generally associated with poor outcomes, even in patients on HAART [139, 140]. The risk of CD8+ T-cell exhaustion is magnified in HIV-hrHPV coinfection, with one study showing that combined chronic inflammation from both viruses leads to exhaustion even in patients on HAART. This reduces HPV clearance in infected cells, allowing for the development of dysplastic squamous lesions and progression to malignancy [71].

Fig. 3. Chronic HIV infection induces widespread chronic inflammation in the human body. This inflammation results in increased PD-1 expression on CD8+ T cells in an attempt to decrease constitutively active systemic immune response. Since CD8+ T cells infiltrate dysplastic and malignant lesions and are crucial to the antitumor response, this increased PD-1 expression (secondary to chronic HIV infection) allows PD-L1-expressing tumors to escape the antitumor response in HIV-infected patients. HIV, human immunodeficiency virus.

Within anal precancerous lesions, HIV coinfection has been shown to reduce CD8+ T-cell infiltration into lesion stroma, resulting in treatment resistance [141]. However, in one study on anal SCC, HIV was not associated with decreased CD8+ T lymphocyte tumor infiltration although the degree of CD8+ T lymphocyte infiltration was shown to significantly predict PD-L1 expression on tumor cells [142]. Another study showed that, in women on HAART, HIV decreased dendritic cell density in anal mucosa as part of the HPV-induced immune response [143]. HIV may also upregulate PD-L1 on dendritic cells, thus decreasing their efficacy in the anti-HPV immune response and further highlighting the interaction between HIV and the PD-1/PD-L1 axis [144]. Other mechanisms have been proposed to explain the increased risk of anal SCC in HIV-HPV coinfecting patients, with one study proposing that HIV induced the overexpression of FoxP3 in regulatory T cells and promoted the depletion of local dendritic cells [145]. Although recent research is promising, further studies are needed on the effect of HIV on

tumor-infiltrating CD8+ T cells, the activation of the PD-1/PD-L1 axis, and other immunologic mechanisms involved in the oncogenicity of HIV-HPV coinfection and anal precancerous lesions.

Implications for Immunotherapy

Investigation into the relationship between CD8+ T cells and PD-1/PD-L1 signaling in HIV-infected patients is potentially valuable for the development of immunotherapies for anal SCC. Of particular relevance is the role of PD-1/PD-L1 blocking agents in anal SCC, and their effects on CD8+ T-cell infiltration. As previously mentioned, the current literature supports CD8+ T-cell exhaustion as a result of chronic HIV infection, mediated by increased PD-1 expression. Also supported is the reduction of immune activity of other cell types, such as dendritic cells, by a similar mechanism. In this context, PD-1 blockade could theoretically ameliorate this exhaustion

and improve the ability of CD8+ T cells to infiltrate tumors and exert cytotoxic activity [146].

The use of PD-1 blocking agents has also been applied to treat HIV infection itself. HIV-related T-cell exhaustion due to chronic infection not only reduces immune response to cancer, precancerous lesions, or other virally infected cells but reduces the immune response to HIV [147]. PD-1 blocking agents have been utilized in this context and were shown in one study to improve HIV-specific immunity, though only 8 participants were included, necessitating further research [148]. The use of PD-1 blockade in cancer has been widely studied and one PD-1 blocking agent, the monoclonal antibody pembrolizumab, is FDA-approved for use in a wide variety of PD-L1-expressing tumors, agnostic of cell of origin. This underscores the established importance of the PD-1/PD-L1 axis on cancer progression and treatment [149, 150]. Several studies have supported the utilization of a threshold for PD-1 expression on CD8+ T cells as an indication for the utility of PD-1 blocking agents in cancer, and there is evidence that PD-1 blockade improves CD8+ T-cell infiltration of tumors [141, 151–154].

The role of PD-1 blockade in anal SCC has also been examined, with several active clinical trials (NCT03233711, NCT02314169, NCT02919969) testing the use of pembrolizumab and nivolumab in patients with anal SCC. Pooled results from the KEYNOTE-028 and KEYNOTE-158 studies showed promising antitumor efficacy in pre-treated anal SCC, as well as a manageable adverse event profile [155, 156]. However, these studies have not specifically explored the efficacy of PD-1 blockade in those with HIV-HPV coinfecting anal SCC. With the increased burden that HIV places on CD8+ T-cell exhaustion via PD-1 expression and the significance of the PD-1/PD-L1 axis in HIV itself, there may be improved efficacy in HIV-infected individuals with anal SCC.

Conclusion

CD8+ T lymphocyte tumor infiltration and its interplay with the PD-1/PD-L1 axis are promising areas of current and future research and may yield effective immunotherapies in HIV-HPV coinfecting individuals with anal SCC. Although there is a growing body of evidence regarding the immunologic modifications conferred by HIV coinfection in the development and progression of anal SCC, further studies are needed to fully elucidate the immune mechanisms underlying this relationship. In parallel with the epidemiologic investigation that has shown an increasing incidence of HPV-associated anal SCC in HIV-infected individuals, future basic science and translational research is warranted to more completely define the interplay between HIV, HPV, and the immune milieu of the anal mucosa, and how they directly impact oncologic risk. Finally, the unique potential role of PD-1 blockade in HPV-mediated anal SCC is promising and warrants further investigation.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

All the authors contributed to this manuscript and reviewed and approved the article.

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