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GASTROINTESTINAL CANCERS (J MEYER, SECTION EDITOR)

Immunotherapy in Anal Cancer

Lisa Phuong [1] · Lakshmi Rajdev [1]

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Abstract

Purpose of Review Standard treatment for early-stage squamous cell cancer of the anal canal (SCCA) includes concurrent chemotherapy and radiation to achieve curative intent. Treatment options are limited, however, especially with locoregional disease relapse occurring in 20–30% of patients and about 10–30% of patients presenting with metastatic disease. With more than 90% of SCCAs occurring in the setting of HPV, immune-based therapies are now the target of possible new treatments for this rare disease. This review highlights the role of immunotherapy in HPV-associated SCCA.

Recent Findings Immunotherapy has been shown to extend progression-free survival and overall survival in various solid malignancies, including SCCA. So far, single-agent monotherapy with either nivolumab or pembrolizumab has shown durable clinical response with a tolerable side effect profile.

Summary The 2018 NCCN guidelines now advise nivolumab or pembrolizumab monotherapy as second-line treatment in the management of metastatic SCCA. Further investigation with immunotherapy continues to be critical for such a rare malignancy with few treatment options.

Keywords Immunotherapy · Anal cancer · Checkpoint inhibition · HPV · Squamous cell cancer of the anal canal

Introduction

Anal cancer is fairly uncommon, making up 0.5% of all new cases in the USA in 2019 and accounting for 2.7% of all gastrointestinal malignancies [1]. However, with more than 8000 estimated new cases and 1250 estimated new deaths in 2019, the incidence and mortality rates have been increasing by 2.2% and 3.1%, respectively, per year over the past 10 years. There are 3 histologic subtypes of anal cancer: squamous cell cancers are the most common, followed by adenocarcinomas and neuroendocrine tumors. At least 90% of squamous cell cancers of the anal canal (SCCAs) are associated with high-risk oncogenic subtypes of human papillomavirus (HPV) [2]. HPV-16 and HPV-18 are the most common HPV subtypes in SCCAs, with HPV-16 occurring in greater than 85% of cases and HPV-18 in about 7% of cases [3]. It is important to note, however, that not all patients with HPV develop SCCA; rather, decreased immunity is an important risk factor for the development of HPV-positive SCCAs [4]. In an immunocompromised state (e.g., HIV/AIDs, autoimmune disease, organ transplant patients on immunosuppressive medications), HPV is not sufficiently cleared, leading to persistent infection and transformation of the normal anal epithelium into neoplasia followed by malignancy.

Standard treatment for early-stage SCCA includes concurrent chemotherapy and radiation to achieve curative intent [5]. Treatment options are limited, however, especially with locoregional disease relapse occurring in 20–30% of patients and about 10–30% of patients presenting with metastatic disease [6]. According to SEER data (2009–2015), the 5-year overall survival rate for localized SCCA is almost 82% but significantly decreases to 32% in the metastatic setting [1]. The 2019 NCCN Clinical Practice Guidelines in Oncology for Anal Carcinoma recommends concurrent doublet chemotherapy and radiation with mitomycin/5-fluorouracil (5-FU), mitomycin/capecitabine, or 5-FU/cisplatin as first-line therapy for localized disease, whereas 5-FU/cisplatin, carboplatin/paclitaxel, oxaliplatin/leucovorin/5-FU (FOLFOX), or cisplatin/leucovorin/5-FU (FOLFCIS) are first-line in the metastatic setting [7]. In those with stage IV disease, carboplatin and paclitaxel are preferred over 5-FU and cisplatin based on an international multicenter phase II InterACCT trial demonstrating similar response rates, but fewer side effects and longer overall survival in those who received carboplatin and

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✉ Lisa Phuong
Lphuong@montefiore.org

[1] Montefiore Medical Center, Bronx, NY, USA

paclitaxel compared to 5-FU and cisplatin [8]. Current treatment recommendations are associated with significant morbidity, thus affecting quality of life and outcomes.

With more than 90% of SCCAs occurring in the setting of HPV, immune-based therapies are now the target of possible new treatments for this rare disease. This review highlights the role of immunotherapy in HPV-associated anal cancer.

Rationale for Immunotherapy in HPV-Related SCCA

Studies have shown that chemotherapy and radiation play a role in engaging the immune microenvironment of cancer cells. Both treatment modalities cause calreticulin to relocate from inside the tumor cell to its surface, allowing dendritic and other antigen-presenting cells to target it for consumption [9]. Furthermore, after treatment with chemotherapy (e.g., anthracyclines, etoposide, cisplatin) or radiation, cancer cells release alarmin, which is a high-mobility group box 1 protein, that activates the immune response and increases the anti-tumor effect of either chemotherapy or radiation [10]. Given that treatment response is in part due to immune-mediated mechanisms, immunotherapy has been evaluated in the use of HPV-related SCCA.

The primary goal of immunotherapy is to assist the immune system in recognizing and destroying tumor cells. One of the mechanisms by which HPV evades the immune system is by its integration with the host genome, leading to expression of viral oncoproteins E6 and E7. E6 binds to tumor suppressor gene p53, thus preventing apoptosis of tumor cells, while E7 binds to phosphorylated retinoblastoma (Rb) protein, leading to uncontrolled cell growth and division [11]. When these oncoproteins are identified by antigen-presenting cells (APCs), tumor-infiltrating lymphocytes (TILs) are activated.

Programmed cell death protein 1, also known as PD-1, is an immune checkpoint regulator that suppresses T cell inflammatory activity to promote self-tolerance [12]. Tumor cells evade anti-tumor activity by overexpressing immune checkpoint surface receptors, such as programmed death ligand 1 (PD-L1), which bind to PD-1 on immune cells, leading to downregulation of T cells. The development of PD-L1 inhibitors has been shown to enable immune recognition and destruction of tumor cells.

Development of Checkpoint Inhibitors in SCCA

Nivolumab and pembrolizumab are two monoclonal antibodies that target PD-1, subsequently preventing this negative feedback interaction, and have shown efficacy in various solid malignancies, including unresectable or metastatic SCCAs.

The NCI9673 (Nivolumab in Treating Patients With Refractory Metastatic Anal Canal Cancer) study was the first prospective multicenter single-arm phase II trial evaluating the safety and efficacy of nivolumab monotherapy in refractory metastatic SCCA [13••]. Thirty-seven patients with unresectable metastatic SCCA who had progressed on at least 1 prior line of chemotherapy were enrolled. PD-L1 expression was not required for study eligibility. Hepatitis-positive and human immunodeficiency virus (HIV)–positive patients were included if the CD4+ T cell count was greater than 300/mm^3. Patients with any history of or active autoimmune disease were excluded. The primary endpoint was response rate. Nivolumab was administered intravenously every 2 weeks at a dose of 3 mg/kg. After a median follow-up of 10.1 months, 2 patients had complete response and 7 patients had partial response based on Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 criteria (overall response rate (ORR) 24%, 95% CI, 15–33%), as well as 17 patients with stable disease for a disease control rate of 72%. Median duration of response was 5.8 months, progression-free survival (PFS) was 4.1 months (95% CI, 3.0–7.9 months), and median overall survival (OS) was 11.5 months (95% CI, 7.1 months–not reached). Treatment was overall well tolerated. There were less than 20% grade 3 toxicities, which included anemia, fatigue, rash, and hypothyroidism. No grade 4 adverse events were reported. At the time of data cutoff, disease progression occurred in 24 of 37 patients (65%) on nivolumab. However, this analysis is limited by its small sample size of 13 patients. Overall, nivolumab was effective with manageable side effects when used as monotherapy for patients with unresectable, refractory SCCA.

KEYNOTE-028 (Study of Pembrolizumab in Participants With Advanced Solid Tumors) was a multi-cohort phase Ib trial for patients with advanced solid malignancies [14••]. Twenty-four patients with PD-L1-positive (defined as at least 1% cells staining positive for 22C3 antibody) SCCA were enrolled, with primary endpoints being safety and ORR. Approximately 86% of patients received at least 1 prior line of systemic treatment. Pembrolizumab 10 mg/kg was administered intravenously once every 2 weeks for up to 2 years or until progression of disease or unacceptable toxicity. Although no complete responses were noted, 4 patients had partial response by RECIST version 1.1 for an ORR of 17% (95% CI, 5–37%), and 10 patients had stable disease for a disease control rate of 58%. Median PFS was 3.0 months (95% CI, 1.7–7.3 months) and median OS was 9.3 months (95% CI, 5.9–not reached). As in the aforementioned study with nivolumab, there were also less than 20% grade 3 toxicities, with the most common adverse events being diarrhea, fatigue, and nausea. There were no treatment-related deaths or discontinuations. Overall, pembrolizumab showed antitumor activity in PD-L1–positive advanced SCCA, as well as a tolerable side effect profile. These encouraging findings have therefore led to continued

investigation of pembrolizumab in advanced SCCA. A multicenter phase II study evaluating ORR in pembrolizumab (200 mg every 3 weeks) for refractory metastatic SCCA is currently enrolling patients (NCT02919969).

With these promising results, the 2018 NCCN guidelines were modified to recommend nivolumab or pembrolizumab monotherapy as second-line treatment in the management of metastatic SCCA.

Biomarker Analysis for Response to Checkpoint Inhibition

Disease response to immune checkpoint inhibition is directly correlated with mutational burden. Patients with metastatic or nonmetastatic SCCA demonstrate low tumor mutational burden, which is consistent with other HPV-related malignancies, including cervical as well as head and neck cancers [15]. Biomarkers were compared from HPV-positive responders and HPV-positive non-responders in the NCI9673 trial to determine which particular patient subset might benefit from the anti-tumor activity of immunotherapy. Compared to non-responders, HPV-positive responders were noted to have an increased number of T cells expressing CD8 and granzyme B, as well as higher PD-1 expression, suggesting that these patients are highly likely to benefit from immune checkpoint inhibition [16]. PD-L1 expression was analyzed from pretreatment tissue samples, and it was noted that higher PD-L1 expression was found in 4 patients who demonstrated response compared to 9 patients who did not respond to treatment.

T Cell Receptor Therapy

Higher tumor-infiltrating lymphocytes (TILs) are correlated with better outcomes in SCCAs. In a retrospective study of approximately 280 patients, there was almost a 30% difference in relapse-free rate between those with high TIL scores versus absent or low TIL scores (92% versus 63%, respectively) [17].

Autologous tumor-specific T cells are returned to the patient after they are expanded ex vivo and undergo myeloablative conditioning and IL-2 costimulation [16]. Nine patients with locally advanced or metastatic, platinum-refractory, HPV-16 or HPV-18 cervical cancer were treated with autologous HPV TILs [18]. One patient demonstrated partial response. Two others had complete response, even after follow-up of 46 and 54 months, respectively. The most common AEs included myelosuppression and infection. Tissue analysis from 2 patients with complete response revealed that TIL recognition of somatic mutations, including SETDB1, METTL17, and ALDH1A1, was more frequent than recognition of HPV viral antigens, including HPV oncoproteins E6 or E7. These TILs were more common within PD-L1-positive T-cells compared to PD-L1-negative T-cells, remaining at high levels even during remission. Given the response in HPV-positive cervical cancer, treatment with anti-PD-1 inhibitors and HPV tumor-infiltrating lymphocytes (TILs) for HPV-positive SCCA appears to be promising.

In another study of 4 patients with HPV-positive metastatic anal cancer, genetically engineered T cells were created to identify the HLA-A 02:01 epitope of the HPV-16 E6 antigen [19]. Two patients had partial response to treatment, lasting 3 and 6 months, respectively. E6 T cell receptor therapy was generally well tolerated. No autoimmune adverse events or toxicities were reported. This further illustrates the promising effects of treatment with T cells. Hence, there is an ongoing trial evaluating E7 T cell receptor therapy with or without anti-PD-1 therapy (NCT02858310).

Immune Vaccinations

The ADXS11-011 vaccine is a live attenuated *Listeria monocytogenes* vector modified to produce peptides that release HPV oncoprotein E7 into the bacterial cytoplasm and was created to evaluate its response against HPV-associated malignancies [20]. Phagocytosis of Listeria results in immune response, leading to lysis of the E7 peptide as well as further activation of E7-specific T cells and antibody production, allowing immune activation against HPV-positive tumor cells.

ADXS11-011 showed promising results in 26 patients with incurable cervical squamous cell carcinoma or adenocarcinoma [21]. One patient had radiologic response, while 9 patients had stable disease. Treatment-related adverse events included hypersensitivity infusion-related reactions, such as fever, chills, and fatigue. Given good response with a tolerable safety profile, a single-arm phase II study of ADXS11-011 monotherapy was evaluated in 29 patients with metastatic SCCA [22•]. One patient had partial radiologic response, while 7 patients had stable disease. The disease control rate was 28%. Treatment-related adverse events were hypersensitivity infusion-related reactions. ADXS11-011 was also studied in 10 patients with locally advanced HPV-positive SCCA receiving concurrent chemotherapy (5-FU and mitomycin C) and radiation [23]. Eight patients had complete response on sigmoidoscopy at 6 months. Eighty-nine percent of these patients remained disease-free at a median follow-up of 34 months. Side effects were manageable, with only 2 patients having grade 3 toxicities, including chills, back pain, or hyponatremia.

The phase 2 FAWCETT trial is evaluating ADXS11-011 monotherapy in patients with relapsed or refractory metastatic SCCA who had previously received at least one prior line of systemic treatment. This study is no longer enrolling patients, and data is currently being analyzed with the primary endpoint being ORR (NCT02399813).

These clinical studies further illustrate the potential benefit of immunotherapy in improving outcomes in SCCA.

HPV-Associated SCCA in HIV/AIDS

HPV-related SCCA is especially prevalent in those with HIV/AIDS, and its incidence continues to rise in the setting of antiretroviral treatments [24]. Currently, secondary prevention programs are being created to prevent anal cancer in this specific population. Although anal cancer recurrence and overall survival rates are similar in those with HIV/AIDS compared to those without HIV/AIDS, treatment toxicities experienced by those with HIV/AIDS tend to be more severe.

Clinical trials traditionally exclude patients with HIV/AIDS; therefore, safety and efficacy of immune checkpoint inhibitors are not well known in this population. A systematic review of 73 patients with HIV/AIDS demonstrated that there was no change in the safety profile of immune checkpoint inhibition in advanced-stage cancer in this population [25•]. Therefore, immune checkpoint inhibitors could certainly be a safe option for those with HIV/AIDS. Additional clinical trials would be beneficial in studying safety and efficacy of immune checkpoint inhibitors in SCCA.

Future Directions

Immunotherapy has been shown to extend progression-free survival and overall survival in various solid malignancies, including SCCA. So far, single-agent monotherapy with either nivolumab or pembrolizumab has shown durable clinical response with a tolerable side effect profile.

Additional studies are underway, including those involving combination immunotherapy, such as anti-CTLA-4 therapy with anti-PD-1 therapy (Table 1). A multi-center phase 2 study evaluating combination nivolumab with or without ipilimumab in refractory metastatic SCCA is currently enrolling patients (NCT02314169) with a primary endpoint of PFS. Furthermore, the HIV Consortium is investigating combination nivolumab with ipilimumab in those with HIV with a CD4 count of at least 200 cells/mm^3 (NCT02408861).

The NCI EA2165 study (NCT03233711) is a phase 2 trial evaluating nivolumab for 6 months after combination chemotherapy (5-FU or capecitabine plus mitomycin-C, or 5-FU plus cisplatin) and radiation in high-risk locally advanced stage 2-3B SCCA with primary endpoint being disease-free survival at 2 years. Patients will be stratified based on nodal status, HIV status, and RT dose.

The NCI9673 (Nivolumab in Treating Patients With Refractory Metastatic Anal Canal Cancer) study showed that PD-1-positive tumors with coexpression of TIM-3 AND LAG3 had durable response to nivolumab. Checkmate358 is an international phase 1/2 trial examining nivolumab monotherapy and nivolumab in combination with ipilimumab, the anti–lymphocyte activation gene 3 (LAG3) agent BMS-986016, or the anti-CD38 agent daratumumab for advanced virus-associated tumors, including anal SCCA, is currently enrolling patients (NCT02488759).

Analysis of anti-VEGF and anti-EGFR therapies in combination with PD-1 therapies are also ongoing. A phase II trial evaluating bevacizumab with atezolizumab in metastatic HPV-associated cancers, including SCCA, is ongoing. Patients with metastatic SCCA who received at least one prior therapy, with no prior immune therapies as part of their treatment regimen, are enrolled to receive bevacizumab 15 mg/kg and atezolizumab 1200 mg IV every 3 weeks. Primary endpoint is response rate based on RECIST criteria, version 1.1 (NCT03074513). In addition, cetuximab and avelumab are

Table 1 Ongoing trials evaluating immunotherapy in SCCA

Identifier (phase)	Patients, *n* (estimated)	Protocol	Setting	Primary endpoint
NCT02314169 (2)	137	Arm I: nivolumab Arm II: nivolumab + ipilimumab	Refractory metastatic SCCA (2L+)	PFS
NCT02408861 (1)	96	Nivolumab + ipilimumab	HIV-associated relapsed or refractory metastatic or unresectable classical Hodgkin lymphoma or solid tumors (1 L)	MTD of nivolumab
NCT03233711 (2)	200	Arm I: nivolumab Arm II: observation	High risk stage II–IIIB anal cancer (1L)	DFS
NCT02488759 (1/2)	1100	Arm I: nivolumab Arm II: nivolumab + ipililumab Arm III: nivolumab + relatlinib Arm IV: nivolumab + daratumumab	Virus-Associated Tumors, including SCCA (1 L)	Safety and tolerability
NCT03074513 (2)	160	Atezolizumab + bevacizumab	Metastatic SCCA (2L+); rare solid tumors	ORR
NCT03944252 (2)	54	Arm I: avelumab Arm II: avelumab + cetuximab	Metastatic or unresectable, locally advanced SCCA (2 L+)	ORR
NCT03519295 (2)	99	Arm I: mDCF + atezolizumab Arm II: mDCF	Metastatic or unresectable, locally advanced recurrent SCCA (1L)	PFS
NCT04046133 (1b/2)	50	Pembrolizumab	Stage IIIA or IIIB SCCA (1L)	Safety and tolerability

being compared to avelumab monotherapy in refractory locally advanced or metastatic SCCA (NCT03944252).

Furthermore, the phase II SCARCE study is evaluating chemotherapy (docetaxel, cisplatin, and 5-FU) with or without atezolizumab in advanced SCCA (NCT03519295).

In the UK, the CoRInTH study is a phase 1b/2 trial evaluating pembrolizumab (administered every 3 weeks for 8 cycles) with concurrent chemotherapy (mitomycin plus 5-FU or capecitabine) and intensity-modulated RT (IMRT) in HPV-positive stage 3 or 4 SCCA (NCT04046133). The trial is designed to compare the safety and efficacy of pembrolizumab given at 3 different points of therapy, at week 5 day 1, week 3 day 1, or week 1 day 1 of chemoradiation.

Conclusions

SCCA is a rare malignancy commonly associated with HPV-16 and HPV-18. Although the HPV vaccination is available and effective as a means of primary prevention for HPV infection and associated malignancies, it is not widely used. The incidence of SCCA continues to slowly rise each year. The current standard of care for locally advanced SCCA includes concurrent chemotherapy and radiation, which unfortunately, has significant morbidity. Furthermore, disease relapse with widespread metastases is not uncommon.

Immune checkpoint inhibition appears to have a promising role in improving outcomes of SCCA. Given the current findings with anti-PD-1 therapies in SCCA with a well-tolerated side effect profile, further investigation for novel approaches as well as potential biomarkers are critical for such a rare malignancy with few treatment options.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Howlader N, Noone AM, Krapcho M, Miller D, Brest A, Yu M, Ruhl J, Tatalovich Z, Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA. SEER Cancer Statistics Review, 1975-2016, National Cancer Institute. Bethesda, MD, https://seer.cancer.gov/csr/1975_2016/, based on November 2018 SEER data submission, posted to the SEER web site, April 2019.
2. Serup-Hansen E, Linnemann D, Skovrider-Ruminski W. Human papillomavirus genotyping and p16 expression as prognostic factors for patients with American Joint Committee on Cancer stages I to III carcinoma of the anal canal. J Clin Oncol. 2014;32:1812–7. https://doi.org/10.1200/JCO.2013.52.3464.
3. Morris VK, Rashid A, Rodriguez-Bigas M, Das P, Chang G, Ohinata A, et al. Clinicopathologic features associated with human papillomavirus/p16 in patients with metastatic squamous cell carcinoma of the anal canal. Oncologist. 2015;20(11):1247–52. https://doi.org/10.1634/theoncologist.2015-0091.
4. Scholefield JH, Castle MT, Watson NF. Malignant transformation of high-grade anal intraepithelial neoplasia. Br J Surg. 2005;92(9):1133–6. https://doi.org/10.1002/bjs.4994.
5. Flam M, John M, Pajak TF, Petrelli N, Myerson R, Doggett S, et al. Role of mitomycin in combination with fluorouracil and radiotherapy, and of salvage chemoradiation in the definitive nonsurgical treatment of epidermoid carcinoma of the anal canal: results of a phase III randomized intergroup study. J Clin Oncol. 1996;14:2527–39. https://doi.org/10.1200/JCO.1996.14.9.2527.
6. Gunderson LL, Winter KA, Ajani JA. Long-term update of US GI intergroup RTOG 98-11 phase III trial for anal carcinoma: survival, relapse, and colostomy failure with concurrent chemoradiation involving fluorouracil/mitomycin versus fluorouracil/cisplatin. J Clin Oncol. 2012;30:4344–51. https://doi.org/10.1200/JCO.2012.43.8085.
7. National Comprehensive Cancer Network (NCCN). NCCN Clinical Practice Guidelines in Oncology. Anal Carcinoma Version 1.2020. 2019 Nov 19;National Comprehensive Cancer Network. Abstract available at https://www.nccn.org/professionals/physician_gls/pdf/anal.pdf.
8. Rao S, Sclafani F, Eng C, Grønlie Guren M, Adams RA, Benson A, et al. InterAACT: a multicentre open label randomised phase II advanced anal cancer trial of cisplatin (CDDP) plus 5-fluorouracil (5-FU) vs carboplatin (C) plus weekly paclitaxel (P) in patients (pts) with inoperable locally recurrent (ILR) or metastatic treatment naïve disease - an International Rare Cancers Initiative (IRCI) trial. Ann Oncol. 2018;29:viii715–6. https://doi.org/10.1093/annonc/mdy424.022.
9. Obeid M, Tesniere A, Ghiringhelli F, Fimia GM, Apetoh L, Perfettini JL, et al. Calreticulin exposure dictates the immunogenicity of cancer cell death. Nat Med. 2007 Jan;13(1):54–61. https://doi.org/10.1038/nm1523.
10. Apetoh L, Ghiringhelli F, Tesniere A, Obeid M, Ortiz C, Criollo A, et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. Nat Med. 2007 Sep;13(9):1050–9. https://doi.org/10.1038/nm1622.
11. Werness BA, Levine AJ, Howley PM. Association of human papillomavirus types 16 and 18 E6 proteins with p53. Science. 1990;248(4951):76–9. https://doi.org/10.1126/science.2157286.
12. Fife BT, Bluestone JA. Control of peripheral T-cell tolerance and autoimmunity via the CTLA-4 and PD-1 pathways. Immunol Rev. 2008;224:166–82. https://doi.org/10.1111/j.1600-065X.2008.00662.x.
13. •• Morris VK, Salem ME, Nimeiri H. Nivolumab for previously treated unresectable metastatic anal cancer (NCI9673): a multicentre, single-arm, phase 2 study. Lancet Oncol. 2017;18:446–53. https://doi.org/10.1016/S1470-2045(17)30104-3. **This was the first prospective multicenter single-arm phase II trial evaluating immunotherapy in SCCA and showed that nivolumab monotherapy was effective and well-tolerated in refractory metastatic SCCA.**
14. •• Ott PA, Piha-Paul SA, Munster P. Safety and antitumor activity of the anti-PD-1 antibody pembrolizumab in patients with recurrent carcinoma of the anal canal. Ann Oncol. 2017;28:1036–41. https://

doi.org/10.1093/annonc/mdx029. **This multi-cohort phase Ib trial evaluated pembrolizumab for patients with advanced solid malignancies, showing antitumor activity in PD-L1–positive advanced SCCA, as well as a tolerable side effect profile. These encouraging findings have therefore led to continued investigation of pembrolizumab in advanced SCCA.**

15. Morris V, Rao X, Pickering C. Comprehensive genomic profiling of metastatic squamous cell carcinoma of the anal canal. Mol Cancer Res. 2017;15:1542–50. https://doi.org/10.1158/1541-7786.MCR-17-0060.
16. Hinrichs CS, Rosenberg SA. Exploiting the curative potential of adoptive T-cell therapy for cancer. Immunol Rev. 2014;257(1):56–71. https://doi.org/10.1111/imr.12132.
17. Gilbert DC, Serup-Hansen E, Linnemann D, Høgdall E, Bailey C, Summers J, et al. Tumour-infiltrating lymphocyte scores effectively stratify outcomes over and above p16 post chemo-radiotherapy in anal cancer. Br J Cancer. 2016;114:134–7. https://doi.org/10.1038/bjc.2015.448.
18. Stevanovic S, Draper LM, Langhan MM. Complete regression of metastatic cervical cancer after treatment with human papillomavirus-targeted tumor-infiltrating T cells. J Clin Oncol. 2015;33:1543–50. https://doi.org/10.1200/JCO.2014.58.9093.
19. Hinrichs CS, Doran SL, Stevanovic S. A phase I/II clinical trial of E6 T-cell receptor gene therapy for human papillomavirus (HPV)-associated epithelial cancers [abstract]. J Clin Oncol. 2017;35(Suppl):Abstract 3009. https://doi.org/10.1200/JCO.2017.35.15_suppl.3009.
20. Miles BA, Monk BJ, Safran HP. Mechanistic insights into ADXS11-001 human papillomavirus-associated cancer immunotherapy. Gynecol Oncol Res Pract. 2017;4:9. https://doi.org/10.1186/s40661-017-0046-9.
21. Huh WK, Dizon DS, Powell MA. ADXS11–001 immunotherapy in squamous or non-squamous persistent/recurrent metastatic cervical cancer: results from stage I of the phase II GOG/NRG0265 study [abstract]. J Clin Oncol. 2016; 34(Suppl): Abstract 5516. Doi: https://doi.org/10.1200/JCO.2016.34.15_suppl.5516.
22.• Eng C, Fakih M, Armin M. P2 study of ADXS11–001 immunotherapy in patients with persistent/recurrent, surgically unresectable locoregional, or metastatic squamous cell anal cancer [abstract]. Ann Oncol. 2017;28(Suppl 5):Abstract 537P. https://doi.org/10.1093/annonc/mdx393.063. **This single-arm phase II study of ADXS11–011 monotherapy showed activity, such that it met the pre-defined 6-month PFS rate, and was overall well-tolerated in metastatic SCCA.**
23. Safran H, Leonard KL, DiPetrillo TA. ADXS11–001 Lm-LLO immunotherapy, mitomycin, 5-fluorouracil (5-FU) and intensity-modulated radiation therapy (IMRT) for anal cancer [abstract]. J Clin Oncol. 2017;35(Suppl):Abstract e15072. https://doi.org/10.1200/JCO.2017.35.15_suppl.e15072.
24. Wang CJ, Palefsky JM. HPV-associated anal cancer in the HIV/AIDS patient. Cancer Treat Res. 2019;177:183–209. https://doi.org/10.1007/978-3-030-03502-0_7.
25.• Cook M, Kim C. Safety and efficacy of immune checkpoint inhibitor therapy in patients with HIV infection and advanced stage cancer: a systematic review. *JAMA Oncol*. 2019;5(7):1049–54. https://doi.org/10.1001/jamaoncol.2018.6737. **A systematic review of HIV/AIDS patients demonstrated that there was no change in the safety profile of immunotherapy in advanced-stage cancer. Additional clinical trials would be beneficial in studying safety and efficacy of immune checkpoint inhibitors in SCCA.**