The Promise of Immunotherapy in Anal Squamous Cell Carcinoma: A Novel Approach for an Orphan Disease

Benny Johnson, DO, and Cathy Eng, MD

Dr Johnson is an assistant professor and Dr Eng is a professor in the Department of Gastrointestinal Medical Oncology at the University of Texas MD Anderson Cancer Center in Houston, Texas.

Corresponding author:
Cathy Eng, MD
Department of Gastrointestinal Medical Oncology
The University of Texas MD Anderson Cancer Center
Holcombe Blvd, Unit 426
Houston, TX 77030
Tel: (713) 792-2828
E-mail: ceng@mdanderson.org

Abstract: An estimated 8200 men and women in the United States will receive a diagnosis of squamous cell carcinoma of the anal canal (SCCA) in 2017. Although SCCA is rare, accounting for 2.6% of gastrointestinal cancers, its incidence rate has been steadily increasing over the last few decades in the United States and around the world. More than 90% of cases of SCCA occur in the context of prior human papillomavirus (HPV) infection. To date, preventive vaccinations against HPV remain markedly underutilized. Most patients who have SCCA present with locoregional disease that is cured with chemoradiation. However, metastatic disease develops in 25% of patients. The management of metastatic SCCA is based on single-institutional case series, with no accepted consensus regarding standard of care. Given the complex interplay between the incorporation of HPV DNA into the host cell genome and the oncogenesis of SCCA, immunotherapeutic strategies have become a strong focus of research efforts regarding the management of SCCA. Recently, a phase 2 trial of an anti–programmed death 1 antibody for refractory SCCA has shown positive results. This review summarizes novel immunotherapies that are under active clinical investigation and describes their potential use in the management of metastatic SCCA.

Keywords
Anal cancer, human papillomavirus (HPV), immunotherapy, oncogenesis, programmed death 1 (PD-1), programmed death ligand 1 (PD-L1), squamous cell cancer

Introduction

Squamous cell carcinoma of the anal canal (SCCA) is a rare malignancy, accounting for just 2.6% of all digestive system cancers in 2017.[1] Alarmingly, the annual incidence of SCCA continues to rise both in the United States and around the world.[2] Preventative vaccinations against human papillomavirus (HPV) remain underutilized in adoloscents.[3] An estimated 8200 new cases of anal carcinoma will be diagnosed this year in the United States, with a projected 1100 patients succumbing to the disease.[1] Most patients with a new diagnosis of SCCA present with locally advanced disease that can be cured with definitive concurrent chemoradiation therapy.[4,5]

Unfortunately, systemic disease ultimately develops in 25% of patients.[6,7] Owing to the low incidence of SCCA and the infrequent development of metastatic disease, there remains a paucity of prospective data to guide the management of metastatic SCCA. Current treatment is limited to systemic doublet chemotherapy

Table 1. Risk Factors for the Development of Anal Cancer

• Concomitant autoimmune disease
• Hematologic malignancies
• High lifetime number of sexual partners
• High-grade anal intraepithelial neoplasia
• High-risk sexual behavior (men who have sex with men, anal-receptive intercourse)
• History of cervical, vaginal, or vulvar cancer
• History of sexually transmitted disease
• Human immunodeficiency virus (HIV) infection
• Human papillomavirus (HPV) infection (high-risk subtypes: HPV-16, HPV-18)
• Immunosuppressive therapies following organ transplant
• Tobacco abuse

based primarily on retrospective, single-institutional case series.[8,9] Therefore, novel therapeutic modalities are coveted for this orphan disease. This review highlights how the complex interplay between HPV infection and SCCA oncogenesis results in susceptibility to immunotherapeutic approaches that hold early promise in refractory SCCA.

The Development of Anal Cancer: Risk Factors and Clinical Milieu

The development of anal cancer is linked to several risk factors (Table 1). By far the most common risk factor to date for SCCA is HPV infection.[10,11] HPV infection is a known etiologic factor in squamous cell carcinomas of the head and neck, cervix, vagina, vulva, and penis.[11-13] Of note, more than 95% of patients with a diagnosis of SCCA have concomitant high-risk HPV infection. The most common subtype of HPV in SCCA is HPV-16, which occurs in more than 85% of cases.[10,11,14-16] The second most common subtype of HPV in these patients is HPV-18, which occurs at the much lower rate of 7.2% of cases.[15,16] The presence of a high-risk subtype of HPV plays a critical role in the transformation of normal anal epithelium to carcinoma.

Interestingly, despite the high prevalence of HPV infection in the general population, SCCA does not develop in most patients with HPV infection. Furthermore, one series reported that grade 2 or grade 3 anal intraepithelial neoplasia progressed to SCCA in only 13% of patients over 5 years.[17] Transformation to malignancy has been shown to occur at an elevated rate in immunocompromised patients.[18] Altered immunity impedes the natural cell-mediated immune responses necessary for adequate clearance of HPV infection, as occurs in immunocompetent patients.[13,19] Therefore, a state of altered immunity serves as the clinical milieu predisposing normal anal epithelium to persistent high-risk HPV infection and eventual malignant transformation. This model of cancer progression is established in other HPV-related malignancies, such as cervical cancer; cervical intraepithelial neoplasia and cervical cancer develop in patients with persistent HPV infection at a greatly elevated rate.[13] Additional risk factors for SCCA that are responsible for a clinical milieu of altered immunity include autoimmune disease, high-risk sexual practices, and exposure to immunosuppressive treatment in the context of organ transplantation.[20,21]

HPV-Associated Oncogenesis and Immune Evasion

The integration of HPV double-stranded circular DNA into the host cell genome results in the expression of viral oncoproteins E6 and E7, promoting the oncogenesis of SCCA.[22-24] The HPV genome encodes early (E6, E7) and late (L1) structural protein during the viral life cycle. E6 binds to the tumor suppressor protein p53 and promotes its degradation, thereby preventing apoptosis.[22] E7 effectively binds to phosphorylated retinoblastoma protein (Rb), enhancing cell cycle activity and augmenting DNA synthesis in S phase.[25] L1, an HPV oncoprotein involved late in the viral replication cycle, plays a key role in capsid formation, facilitating continued reinfection.

E6 and E7 oncoproteins are also intricately involved in immune evasion through various other mechanisms.[26,27] Most notable is the "hijacking" of the differentiation system of the keratinocyte, the target cell of HPV.[27,28] As the keratinocyte goes through its life cycle, with the ultimate goal of becoming a terminally differentiated squamous cell, it is physiologically programmed for death.[28] The virus replicates within the keratinocyte and is subsequently released when the cell dies.[28] This is a natural cellular process and no inflammatory markers are released, which allows HPV to persist without alarming the host immune system. This evasion mechanism makes possible continued infection and ongoing oncogenesis.

Additionally, high-risk HPV subtypes inhibit interferon synthesis via mechanisms, mediated by E6 and E7 oncoproteins, that diminish the presentation of viral antigens to the host immune system.[26,27,29,30] Given that E6 and E7 reduce E-cadherin and the transporter associated antigen processing protein 1, respectively—promoting immune evasion through impaired viral antigen presentation—it is imperative to increase our understanding of the viral biology responsible for oncogenesis in HPV-associated

SCCA, and to continue the investigation of novel immunotherapeutic approaches to treatment.[29,30]

Risk Reduction With Preventative Vaccination Against HPV

High-grade anal intraepithelial neoplasia (HGAIN) may be a precursor lesion for anal cancer, with treatment of HGAIN suggestive of cancer preventative benefit.[17,18,31] Although routine screening for HGAIN remains controversial to date, the benefits of HPV vaccination have been realized.[30,31] One study of 602 men 16 to 26 years of age who were having sex with other men were randomly assigned to receive placebo or the quadrivalent HPV vaccine, which includes the high-risk HPV subtypes 16 and 18.[32] Although anal cancer did not develop in any patient in either group during the 3-year follow-up period, significantly fewer cases of precancerous HGAIN developed in the patients in the vaccination arm.[32] Despite these results, fewer than half of male and female adolescents in the United States have received preventative HPV vaccinations.[3,33] Increasing community awareness regarding the importance of vaccination adherence remains paramount in the primary prevention of SCCA.

Immunotherapy Against Malignancy

Enthusiasm for the use of immunotherapy against cancer was renewed in 2011, when the US Food and Drug Administration (FDA) approved the first checkpoint inhibitor, ipilimumab (Yervoy, Bristol-Myers Squibb), for use in advanced melanoma after a phase 3 study showed an impressive improvement in overall survival (OS). Additional prospective trials of immunotherapy resulted in FDA approval of checkpoint inhibitors in 5 refractory malignancies: non–small cell lung cancer, head and neck cancer, bladder cancer, renal cell cancer, and Hodgkin lymphoma.[34-43] These humanized monoclonal antibodies target cytotoxic T-lymphocyte–associated protein 4 (CTLA-4) on the surface of T cells, programmed death 1 (PD-1) on the surface of T cells, or programmed death ligand 1 (PD-L1) on the surface of tumor cells. This process results in the reactivation of effector cytotoxic T cells against the tumor, impairing immune evasion mechanisms achieved through previous "commandeering" of the checkpoint system.[44-47] Further investigation to isolate meaningful biomarkers of response to allow ideal patient selection remains an area of active clinical investigation. Most notably, cumulative data from 149 patients with microsatellite instability–high or mismatch repair–deficient cancers enrolled in 5 uncontrolled, multicenter, multicohort, single-arm clinical trials resulted in the first-ever FDA tissue-agnostic accelerated approval of the anti–PD-1 agent pembrolizumab (Keytruda, Merck) for the treatment of adult and pediatric patients with refractory, unresectable, or metastatic microsatellite instability–high or mismatch repair–deficient cancers and no satisfactory alternative treatment options. This approval represents a landmark paradigm shift in both drug development and the clinical management of refractory cancer with immunotherapy.

Rationale for Immunotherapy in HPV-Associated SCCA

In response to the continued success of immunotherapy in both solid and hematologic cancers, there is obvious excitement regarding its efficacy in virus-associated malignancies such as SCCA. As previously summarized, HPV oncoproteins E6 and E7 are involved in the oncogenesis of SCCA in anal squamous epithelium.[22,48] The HPV oncoprotein L1 facilitates continued reinfection with HPV, whereas the detection of E6 and E7 by antigen-presenting cells promotes host immunity by triggering an antitumor immune response through the recruitment of tumor-infiltrating lymphocytes (TILs).[49-51] To combat the host antitumor immune response, tumor cells augment their immune evasion properties by overexpressing PD-L1. When PD-L1 is bound to its inhibitory receptor PD-1 on the surface of effector cytotoxic T cells, T-cell activation is downregulated.[45,47] Although this tumor microenvironment consisting of HPV viral oncoproteins clearly promotes oncogenesis, the simultaneous recruitment of TILs has been identified as a vulnerability of the tumor to emerging targeted immunotherapies. For instance, when the interaction between PD-1 and PD-L1 is inhibited, tumor manipulation of the immune checkpoint system is significantly impaired, reinitiating T-cell cytotoxicity. Furthermore, targeting HPV-specific viral oncoproteins may provide an additional avenue for success with novel immunotherapeutic modalities.

Checkpoint Inhibition in Metastatic SCCA

Although in most patients with anal cancer locoregional disease is diagnosed that is amenable to cure with concurrent chemoradiation therapy per the Nigro protocol, 10% of patients initially present with stage IV disease, and metastatic disease develops in 10% to 25% of patients treated for locoregional disease.[6] Owing to the rarity of SCCA, no large prospective clinical trials have been conducted to guide the management of metastatic disease. Doublet chemotherapy with carboplatin/paclitaxel or 5-fluorouracil (5-FU)/cisplatin is the mainstay of treatment on the basis of small, single-institutional, retrospective series.[8,9,52] Of note, we are awaiting the results of the international multicenter InterACCT trial (Cisplatin and Fluorouracil Compared With Carboplatin and

Paclitaxel in Treating Patients With Inoperable Locally Recurrent or Metastatic Anal Cancer; NCT02560298), which involves a head-to-head comparison of 2 forms of doublet chemotherapy in the first-line setting for unresectable metastatic SCCA. The primary outcome measure is the overall response rate (ORR), and the anticipated trial completion date is August 2018. Therefore, to date, there remains no consensus regarding the standard of care for patients with metastatic SCCA. It is unsurprising that immunotherapy has become a major focus for this orphan disease, given the underlying HPV-associated oncogenesis of SCCA and the fact that treatment modalities are desperately coveted in the metastatic setting. Pembrolizumab and nivolumab (Opdivo, Bristol-Myers Squibb) are humanized monoclonal antibodies against PD-1. Disruption of the interaction between PD-L1 on tumor cells and PD-1 on cytotoxic T cells overcomes adaptive immune resistance by enabling T-cell cytotoxicity against tumor.

Monoclonal antibodies against PD-1 and PD-L1, including pembrolizumab, nivolumab, and atezolizumab (Tecentriq, Genentech), have shown activity in a wide array of advanced solid and hematologic malignancies.[34-43] The results of the cohort of patients with advanced PD-L1–positive anal carcinoma treated with pembrolizumab in KEYNOTE-028 (Study of Pembrolizumab in Participants With Advanced Solid Tumors), a multicohort, phase 1b trial for patients with advanced solid tumors, were recently reported.[53] PD-L1 expression of at least 1% was required for trial enrollment, and primary endpoints were safety and ORRs. PD-L1 positivity was found in 74% of the patients screened. More than 86% of the patients (20) had received at least 1 prior line of systemic therapy. Pembrolizumab was administered intravenously every 2 weeks at a dose of 10 mg/kg. Among the 24 patients with SCCA (1 patient enrolled had perineal epidermoid carcinoma), no complete responses were noted. However, 4 patients had a confirmed partial response by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1, for an ORR of 17%, and 10 patients (42%) had confirmed stable disease, for a reported disease control rate of 58%.[53] Median progression-free survival (PFS) was 3.0 months (95% CI, 1.7-7.3), and 6- and 12-month PFS rates of 31.6% and 19.7%, respectively, were reported. Median OS was 9.3 months (95% CI, 5.9 to not available), with 6- and 12-month OS rates of 64.5% and 47.6%, respectively.[53] Overall, pembrolizumab demonstrated encouraging antitumor activity in PD-L1–positive advanced SCCA, along with a manageable side effect profile that most commonly included diarrhea, fatigue, and nausea, with no treatment-related deaths or discontinuations. These findings of durable clinical responses in advanced SCCA support the continued investigation of immunotherapy as a primary modality for this disease. A phase 2 study of pembrolizumab with a primary endpoint of ORR for refractory metastatic SCCA is currently recruiting patients (NCT02919969). Key inclusion criteria include prior progression or intolerance of 5-FU and platinum therapy (Table 2).

NCI9673 (Nivolumab in Treating Patients With Refractory Metastatic Anal Canal Cancer) from the National Cancer Institute was the first prospective multicenter phase 2 trial of single-agent nivolumab for patients with previously treated metastatic SCCA.[54] In this trial, patients with unresectable metastatic SCCA that had progressed on at least 1 prior line of chemotherapy (median, 2 lines of therapy) were eligible to enroll. The primary endpoint of the study was response rate with nivolumab monotherapy. A total of 37 patients were enrolled and treated. Nivolumab was administered intravenously every 2 weeks at a dose of 3 mg/kg. Among these patients, 9 had responses by RECIST 1.1 (ORR, 24%). Also noted were 2 complete responses and 7 partial responses. Therapy was well tolerated, with anemia, fatigue, rash, and hypothyroidism the only grade 3 toxicities among a total of 5 patients. Of note, this trial also included human immunodeficiency virus–positive patients with SCCA if the CD4+ T-cell count exceeded 300/mm^3. However, patients with active autoimmune disease or a history of autoimmune disease were excluded. At the time of data cutoff, disease progression had developed in 24 of the 37 patients (65%) while they were on nivolumab. Nonetheless, this is the first prospective clinical study to be completed for patients with previous progression on systemic chemotherapy. Overall, nivolumab was well tolerated and effective when used as monotherapy for patients with unresectable, refractory SCCA. These findings represent the first major breakthrough in the management of anal SCCA in more than 20 years. The results are encouraging in that tumor shrinkage was achieved in nearly 25% of the patients treated with nivolumab. The remaining patients in this study did not benefit from an immunotherapeutic approach, however. Therefore, moving forward, it will be critical to identify biomarkers to select patients for clinical trials incorporating immunotherapy.

To that end, interesting immunohistochemical and flow cytometry biomarker correlates were also reported in this study. Baseline percentages of T cells expressing CD8 and granzyme B were found to be higher in responders to anti–PD-1 therapy than in nonresponders. Responders were also found to have higher concentrations of PD-1 in immune cells of the tumor microenvironment in comparison with nonresponders.[55] Flow cytometry analysis of fresh tumor samples revealed that PD-1 expression on CD8+ T cells was higher for responders than for nonresponders at baseline. Further investigation regarding the use of predictive biomarkers to select patients for anti–PD-1 therapy is needed. Of note, subgroup analysis revealed no significant relationship

Table 2. Immunotherapy-Based Trials for the Treatment of Refractory Metastatic Squamous Cell Carcinoma of the Anal Canal

Identifier (Phase)	N	Immunotherapeutic Trial Agent	Status	Results to Date
NCT02054806 (1b)	24	Pembrolizumab (anti–PD-1 monotherapy)	Completed[54]	ORR, 17% DCR, 58% mPFS, 3.0 mo mOS, 9.3 mo
NCT02314169 (2)	37	Nivolumab (anti–PD-1 monotherapy)	Completed[55]	ORR, 24% mPFS, 4.1 mo mOS, 11.5 mo
NCT02280811 (1/2)	12[a]	E6 TCR T-cell therapy	Completed	Poster at 2017 ASCO annual meeting: partial responses lasting 6 and 3 mo achieved in 2 patients with anal cancer
NCT02399813 (2)	Stage 1: 31 Stage 2: 24	ADXS11-001 (*Listeria*-based immune vaccine)	Accrual completed	Study ongoing
NCT02919969 (2)	32 (estimated)	Pembrolizumab	Actively recruiting	NA
NCT02858310 (1)	180 (estimated)[b]	E7 TCR T cells +/- pembrolizumab	Actively recruiting	NA
NCT02488759 (1/2)	500 (estimated)[c]	Nivolumab +/- ipilimumab (anti–CTLA-4), BMS-986016 (anti-*LAG3*), or daratumumab (anti-CD38)	Actively recruiting	NA

ASCO, American Society of Clinical Oncology; CTLA-4, cytotoxic T-lymphocyte–associated protein 4; DCR, disease control rate; HPV, human papillomavirus; LAG3, lymphocyte activation gene 3; mOS, median overall survival; mPFS, median progression-free survival; N, number of patients; NA, not available; ORR, overall response rate; PD-1, programmed death 1; TCR, T-cell receptor.

[a] Total of 12 HPV-positive patients in cohort: 4 anal, 6 cervical, 1 oropharyngeal, and 1 vaginal.

[b] Includes patients with HPV-associated advanced malignancies: anal, cervical, oropharyngeal, penile, vaginal, and vulvar cancers.

[c] Includes patients with virus-associated advanced tumors in addition to patients with metastatic squamous cell carcinoma of the anal canal (cervical cancer, Epstein-Barr virus–positive gastric cancer, HPV-positive and -negative squamous cell cancer of the head and neck, Merkel cell carcinoma, nasopharyngeal cancer, penile cancer, vaginal cancer, or vulvar cancer).

between sex, prior exposure to platinum chemotherapy, use of radiation therapy, or specific sites of distant metastases and response to anti–PD-1 therapy.[54]

Given the paucity of treatment options for refractory SCCA, these studies of anti–PD-1 therapy highlight the immense promise of novel immunotherapeutic approaches with checkpoint inhibition for this distinct patient population. Of note, owing to the success of dual checkpoint blockade in enhancing survival in advanced melanoma and other solid malignancies, a study of the combination of nivolumab with the anti–CTLA-4 monoclonal antibody ipilimumab is being planned as an extension of the NCI9673 study of refractory SCCA. Additionally, an international phase 1/2 trial that is investigating nivolumab monotherapy and nivolumab in combination with ipilimumab, the anti–lymphocyte activation gene 3 (*LAG3*) agent BMS-986016, or the anti-CD38 agent daratumumab (Darzalex, Janssen) for advanced virus-associated tumors, including anal SCCA, is actively recruiting patients (An Investigational Immuno-therapy Study to Investigate the Safety and Effectiveness of Nivolumab, and Nivolumab Combination Therapy in Virus-associated Tumors [CheckMate358]; NCT02488759; Table 2). Furthermore, an Eastern Cooperative Oncology Group (ECOG) protocol that uses 5-FU or capecitabine, mitomycin-C, and radiation therapy plus or minus nivolumab for 6 months in patients with high-risk (T4 or N+, M0) SCCA is pending final draft.

Future Directions: Innovative Immunotherapeutic Modalities for SCCA

In adoptive T-cell transfer (ACT), T cells specific to an antigen of interest are isolated from a tumor via the ex vivo expansion of tumor-reactive T cells, genetically

engineered, then autologously reinfused as TILs.[55,56] In a trial of patients with refractory metastatic cervical cancer, durable responses were achieved in 2 of 9 patients treated with HPV E6/E7 TIL infusion, and an additional patient had a partial response per RECIST 1.1.[57] Expanding the use of HPV TIL therapy to relapsed or refractory metastatic SCCA is of obvious clinical interest because novel approaches to this orphan disease are desperately needed.

Patient-Centered Genetically Engineered T-cell Therapy

Another component of ACT therapy involves the groundbreaking use of patient-centered genetically engineered T-cell therapy. The patient's blood is collected via blood draw or leukapheresis. T cells are then transfected or transduced with T-cell receptor (TCR) or chimeric antigen receptor (CAR) genes through lentivirus, gamma-retrovirus, or nonviral gene transfer ex vivo.[58] The modified T cells are expanded ex vivo and intravenously administered to circulate through the patient's bloodstream. Upon encountering tumor, they bind antigen and initiate the destruction of tumor cells.[58] The critical component of successful T-cell therapy for epithelial tumors involves the targeted antigen. In prior experience that focused on antigens shared by tumor and healthy tissue, treatment was limited by the ensuing toxicity.[59,60] Given the exclusivity of E6 and E7 oncoproteins to HPV-positive cells and their absence in healthy tissues, patients with HPV-positive tumors may be ideal candidates for T-cell therapy.

TCR therapy. Previous preclinical work with an excised metastatic anal tumor sample investigated the use of TCR therapy to target HPV-positive tumor cells. In this study, after T cells were genetically engineered to recognize an HLA-A*02:01-restricted epitope of HPV-16 E6, HPV-16–positive head and neck and cervical cancer cell lines were successfully targeted and killed.[61] These novel findings provided the foundation for an early-phase clinical trial in which HPV-16–positive tumors were targeted with T cells genetically engineered to be specific for E6 TCRs.[61] The National Cancer Institute recently completed a phase 1/2 study in patients with metastatic, refractory, or recurrent HPV-16–positive cancer (anal, cervical, oropharyngeal, penile, vaginal, or vulvar) that used TCR gene therapy targeting HPV-16 E6 (T Cell Receptor Immunotherapy Targeting HPV-16 E6 for HPV-Associated Cancers; NCT02280811; Table 2). Results for the 12 treated patients, 4 of whom had anal cancer, have been released in abstract form.[62] The only 2 partial responders in this trial had anal cancer, and their responses lasted for 6 and 3 months after treatment.[62] The patient with the 6-month response was subsequently deemed a candidate for metastasectomy and had no evidence of disease 22 months after undergoing surgery. Although the levels of E6 TCR T-cell memory were maintained in the responding patients over time, the levels in nonresponders ranged widely. This finding suggests that other immunologic factors play a role in the effect of treatment. Of note, E6 TCR T-cell therapy was well tolerated, with no cytokine storm, autoimmune adverse events, or dose-limiting toxicities reported. Furthermore, in light of the success to date with checkpoint inhibition therapy, clinical trial investigation of TCR gene therapy alone and in combination with anti–PD-1 therapy is being pursued at the NCI (E7 TCR T Cells With or Without PD-1 Blockade for Human Papillomavirus-Associated Cancers; NCT02858310; Table 2).

CAR T-cell therapy. This is a novel therapy that targets cancer cells with patient-specific, genetically engineered T cells. Unlike TCR gene therapy modalities, CAR T-cell therapy is not limited by haplotype restriction. This allows a benefit in a much larger patient population. Initially developed in 1989, first-generation CARs involved a TCR stimulatory domain associated with a single-chain variable antibody fragment.[63,64] Second-generation CARs include a costimulatory domain, which significantly augments success by improving T-cell survival and proliferation when antigen-expressing target cells are engaged.[65,66] In patients with relapsed or refractory B-cell malignancies, the use of CAR-modified T cells targeting the B-cell receptor CD19 have produced durable remissions.[67-70] For instance, among heavily pretreated patients with B-cell acute lymphocytic leukemia, treatment with CARs has demonstrated unparalleled complete remission rates of 80% to 90%.[70] The ability of this practice-changing modality to produce durable responses and impressive survival rates in refractory hematologic malignancies has resulted in FDA approval of tisagenlecleucel (Kymriah, Novartis), the first gene therapy to receive approval in the United States. This approval has generated significant enthusiasm about the potential application of CAR T-cell therapy in solid cancers.

Although it has not yet reached the degree of success realized in hematologic malignancies, extensive clinical investigation of CAR T-cell therapy for solid malignancies continues. Its use in neuroblastoma is the most notable to date.[71,72] For gastrointestinal cancers, numerous early-phase clinical trials are being conducted in the United States and internationally that are investigating the use of CAR T cells in pancreatic cancer.[64] Owing to various tumor-specific antigens in pancreatic cancer—such as mesothelin, carcinoembryonic antigen, prostate stem cell antigen, human epidermal growth factor receptor 2 (HER2), MUC1, and

CD133—CAR T-cell therapy may be an ideal approach for the treatment of this disease.[64] Given the link between SCCA and HPV-associated oncogenesis, tumor cell surface viral proteins such as E6 and E7 conceptually represent exceptional candidate antigens for the use of CAR T-cell therapy because these oncoproteins are absent in healthy human tissues. Therefore, clinical trials investigating HPV-16 E6 and E7 CAR T cells are eagerly anticipated.

Listeria-*Based Immune Vaccines*

A phase 2 trial previously reported the experience of using a novel immunotherapy vaccine, ADXS11-001, with or without cisplatin to treat refractory metastatic cervical cancer.[73] ADXS11-001 is a live attenuated *Listeria monocytogenes* bacterium bioengineered to express a fusion Lm-LLO-E7 protein.[74] Dendritic cells recognize this fusion protein as foreign, and phagocytosis results in the release of peptide fragments of the fusion as tumor-specific antigens that promote the activation of cytotoxic T cells to target E7. This immunotherapeutic approach resulted in an overall disease control rate of 43% (47/110 patients). While in the study, 35 patients had stable disease. There were 6 patients who achieved a complete response and 6 patients who had a partial response, consistent with a response rate of 11%. The response appeared to be durable, given that 28% of the patients were alive at 18 months. The vaccine was well tolerated overall, with only 2% of patients experiencing grade 3 toxicities. Adding cisplatin to ADXS11-001 did not increase efficacy. Of note, activity with ADXS11-001 was appreciated among various high-risk HPV strains.

The phase 2 FAWCETT trial (Phase 2 Study of ADXS11-001 in Subjects With Carcinoma of the Anorectal Canal; NCT02399813) is evaluating ADXS11-001 monotherapy, also known as axalimogene filolisbac, in patients with relapsed or refractory metastatic SCCA of the anorectal canal who have received at least 1 prior therapy. This trial has finished enrolling patients, and the initial results are expected in 2018 (Table 2). Key endpoints of the study include ORR per RECIST (primary) and immune RECIST (secondary), as well as 6-month PFS. This is a 2-stage Simon study, with the potential use of combination checkpoint inhibitor therapy in the second stage.

A phase 1/2 clinical trial evaluated the safety and efficacy of ADXS11-001 with concurrent standard chemoradiotherapy (CRT) consisting of mitomycin, 5-FU, and radiation therapy for HPV-associated locally advanced SCCA.[75] The objective of this study was to determine the safety of ADXS11-001 with CRT and obtain preliminary data on PFS in locally advanced anal cancer. A complete response was noted on sigmoidoscopy at 6 months in 8 of the 10 patients, with 89% of patients disease-free at a median follow-up of 34 months.[75] Only 2 patients had grade 3 toxicities, which consisted of chills, back pain, or hyponatremia. Overall, ADXS11-001 was safe in combination with CRT for SCCA. Given the high cure rates achieved with standard CRT alone for locally advanced SCCA, it is too soon to quantify the additional benefit that ADXS11-001 may provide. The RTOG Foundation is creating a trial of 5-FU/mitomycin-C/radiotherapy with or without ADXS11-001 in patients with high-risk (T >4 cm, N+M0), locally advanced anal cancer. Further investigation is warranted regarding the immune-mediated effects of currently used cytotoxic agents and radiation, and how these mechanisms might be exploited in combination CRT-immunotherapy approaches—along with the importance of sequencing these agents in multidrug regimens.

Conclusions

Although SCCA is responsible for fewer than 3% of gastrointestinal malignancies, the incidence continues to rise in the United States and around the world. To date, SCCA remains an orphan disease with no consensus guidelines regarding the management of refractory metastatic disease. Current recommendations for cytotoxic doublet chemotherapy stem primarily from single-institutional experiences pending the results of ECOG EA2133/InterACCT. Therefore, heavily pretreated patients comprise a distinct group in dire need of novel treatment modalities. The complex interplay between HPV infection, viral oncoproteins, and subsequent SCCA oncogenesis suggests potential tumor vulnerability to immunotherapy. Preliminary findings with anti–PD-1 therapy alone for patients with refractory SCCA are encouraging, although ongoing analysis of biomarker expression for appropriate patient selection will be crucial moving forward. Further investigation using a checkpoint inhibitor approach combined with anti–PD-1 and anti–CTLA-4 therapy is planned. Additionally, patients with HPV-positive SCCA are ideal candidates for investigational immunotherapeutic approaches, such as patient-centered genetically engineered T-cell therapy (CAR and TCR), TILs, and *Listeria*-based immune vaccines owing to the exclusivity of E6 and E7 oncoproteins to HPV-positive cells and their absence in healthy tissues. The combination of these novel approaches with anti–PD-1 therapy is attractive and is being pursued at various academic centers. Given the current scarcity of treatments for metastatic SCCA, ongoing investigation with innovative immunotherapy-based approaches will remain a critical focus of protocol development in the years ahead.

Disclosures

The authors have no financial disclosures.

References

1. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. *CA Cancer J Clin*. 2017;67(1):7-30.
2. Viens LJ, Henley SJ, Watson M, et al. Human papillomavirus-associated cancers - United States, 2008-2012. *MMWR Morb Mortal Wkly Rep*. 2016; 65(26):661-666.
3. Reagan-Steiner S, Yankey D, Jeyarajah J, et al. National, regional, state, and selected local area vaccination coverage among adolescents aged 13-17 years - United States, 2015. *MMWR Morb Mortal Wkly Rep*. 2016;65(33):850-858.
4. Flam M, John M, Pajak TF, 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(9):2527-2539.
5. Bartelink H, Roelofsen F, Eschwege F, et al. Concomitant radiotherapy and chemotherapy is superior to radiotherapy alone in the treatment of locally advanced anal cancer: results of a phase III randomized trial of the European Organization for Research and Treatment of Cancer Radiotherapy and Gastrointestinal Cooperative Groups. *J Clin Oncol*. 1997;15(5):2040-2049.
6. Eng C. Anal cancer: current and future methodology. *Cancer Invest*. 2006;24(5):535-544.
7. Das P, Bhatia S, Eng C, et al. Predictors and patterns of recurrence after definitive chemoradiation for anal cancer. *Int J Radiat Oncol Biol Phys*. 2007;68(3):794-800.
8. Ajani JA, Carrasco CH, Jackson DE, Wallace S. Combination of cisplatin plus fluoropyrimidine chemotherapy effective against liver metastases from carcinoma of the anal canal. *Am J Med*. 1989;87(2):221-224.
9. Faivre C, Rougier P, Ducreux M, et al. [5-fluorouracile and cisplatinum combination chemotherapy for metastatic squamous-cell anal cancer]. *Bull Cancer*. 1999;86(10):861-865. In French.
10. Daling JR, Madeleine MM, Johnson LG, et al. Human papillomavirus, smoking, and sexual practices in the etiology of anal cancer. *Cancer*. 2004;101(2):270-280.
11. De Vuyst H, Clifford GM, Nascimento MC, Madeleine MM, Franceschi S. Prevalence and type distribution of human papillomavirus in carcinoma and intraepithelial neoplasia of the vulva, vagina and anus: a meta-analysis. *Int J Cancer*. 2009;124(7):1626-1636.
12. Gillison ML, Koch WM, Capone RB, et al. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. *J Natl Cancer Inst*. 2000;92(9):709-720.
13. Rodríguez AC, Schiffman M, Herrero R, et al. Longitudinal study of human papillomavirus persistence and cervical intraepithelial neoplasia grade 2/3: critical role of duration of infection. *J Natl Cancer Inst*. 2010;102(5):315-324.
14. Morris VK, Rashid A, Rodriguez-Bigas M, et al. Clinicopathologic features associated with human papillomavirus/p16 in patients with metastatic squamous cell carcinoma of the anal canal. *Oncologist*. 2015;20(11):1247-1252.
15. Hoots BE, Palefsky JM, Pimenta JM, Smith JS. Human papillomavirus type distribution in anal cancer and anal intraepithelial lesions. *Int J Cancer*. 2009;124(10):2375-2383.
16. Palmer JG, Scholefield JH, Coates PJ, et al. Anal cancer and human papillomaviruses. *Dis Colon Rectum*. 1989;32(12):1016-1022.
17. Watson AJ, Smith BB, Whitehead MR, Sykes PH, Frizelle FA. Malignant progression of anal intra-epithelial neoplasia. *ANZ J Surg*. 2006;76(8):715-717.
18. Scholefield JH, Castle MT, Watson NF. Malignant transformation of high-grade anal intraepithelial neoplasia. *Br J Surg*. 2005;92(9):1133-1136.
19. Evander M, Edlund K, Gustafsson A, et al. Human papillomavirus infection is transient in young women: a population-based cohort study. *J Infect Dis*. 1995;171(4):1026-1030.
20. Sunesen KG, Nørgaard M, Thorlacius-Ussing O, Laurberg S. Immunosuppressive disorders and risk of anal squamous cell carcinoma: a nationwide cohort study in Denmark, 1978-2005. *Int J Cancer*. 2010;127(3):675-684.
21. Frisch M, Glimelius B, van den Brule AJ, et al. Sexually transmitted infection as a cause of anal cancer. *N Engl J Med*. 1997;337(19):1350-1358.
22. Werness BA, Levine AJ, Howley PM. Association of human papillomavirus types 16 and 18 E6 proteins with p53. *Science*. 1990;248(4951):76-79.
23. Li X, Coffino P. High-risk human papillomavirus E6 protein has two distinct binding sites within p53, of which only one determines degradation. *J Virol*. 1996;70(7):4509-4516.
24. Balsitis SJ, Sage J, Duensing S, Münger K, Jacks T, Lambert PF. Recapitulation of the effects of the human papillomavirus type 16 E7 oncogene on mouse epithelium by somatic Rb deletion and detection of pRb-independent effects of E7 in vivo. *Mol Cell Biol*. 2003;23(24):9094-9103.
25. Ruiz S, Santos M, Segrelles C, et al. Unique and overlapping functions of pRb and p107 in the control of proliferation and differentiation in epidermis. *Development*. 2004;131(11):2737-2748.
26. Ghittoni R, Accardi R, Hasan U, Gheit T, Sylla B, Tommasino M. The biological properties of E6 and E7 oncoproteins from human papillomaviruses. *Virus Genes*. 2010;40(1):1-13.
27. Stanley M. Immune responses to human papillomavirus. *Vaccine*. 2006;24(suppl 1):S16-S22.
28. Bernardi MP, Ngan SY, Michael M, et al. Molecular biology of anal squamous cell carcinoma: implications for future research and clinical intervention. *Lancet Oncol*. 2015;16(16):e611-e621.
29. Caberg JH, Hubert PM, Begon DY, et al. Silencing of E7 oncogene restores functional E-cadherin expression in human papillomavirus 16-transformed keratinocytes. *Carcinogenesis*. 2008;29(7):1441-1447.
30. Hubert P, Caberg JH, Gilles C, et al. E-cadherin-dependent adhesion of dendritic and Langerhans cells to keratinocytes is defective in cervical human papillomavirus-associated (pre)neoplastic lesions. *J Pathol*. 2005;206(3):346-355.
31. Gautier M, Brochard C, Lion A, et al. High-grade anal intraepithelial neoplasia: progression to invasive cancer is not a certainty. *Dig Liver Dis*. 2016;48(7):806-811.
32. Palefsky JM, Giuliano AR, Goldstone S, et al. HPV vaccine against anal HPV infection and anal intraepithelial neoplasia. *N Engl J Med*. 2011;365(17):1576-1585.
33. Stokley S, Cohn A, Dorell C, et al. Adolescent vaccination-coverage levels in the United States: 2006-2009. *Pediatrics*. 2011;128(6):1078-1086.
34. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med*. 2010;363(8):711-723.
35. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med*. 2012;366(26):2443-2454.
36. Powles T, Eder JP, Fine GD, et al. MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. *Nature*. 2014;515(7528):558-562.
37. Postow MA, Chesney J, Pavlick AC, et al. Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. *N Engl J Med*. 2015;372(21):2006-2017.
38. Robert C, Long GV, Brady B, et al. Nivolumab in previously untreated melanoma without BRAF mutation. *N Engl J Med*. 2015;372(4):320-330.
39. Ansell SM, Lesokhin AM, Borrello I, et al. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. *N Engl J Med*. 2015;372(4):311-319.
40. Motzer RJ, Escudier B, McDermott DF, et al. Nivolumab versus everolimus in advanced renal-cell carcinoma. *N Engl J Med*. 2015;373(19):1803-1813.
41. Brahmer J, Reckamp KL, Baas P, et al. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. *N Engl J Med*. 2015;373(2):123-135.
42. Ferris RL, Blumenschein GR, Fayette J, et al. Further evaluation of nivolumab (nivo) versus investigator's choice (IC) chemotherapy for recurrent or metastatic (R/M) squamous cell carcinoma of the head and neck (SCCHN): Checkmate 141 [ASCO abstract 6009]. *J Clin Oncol* 2016;34(15)(suppl).
43. Perez-Gracia JL, Loriot Y, Rosenberg JE, et al. Atezolizumab (atezo) in platinum-treated advanced or metastatic urothelial carcinoma (mUC): outcomes by prior therapy [ASCO abstract 323]. *J Clin Oncol*. 2017;35(15)(suppl).
44. Schneider H, Downey J, Smith A, et al. Reversal of the TCR stop signal by CTLA-4. *Science*. 2006;313(5795):1972-1975.
45. Ishida Y, Agata Y, Shibahara K, Honjo T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. *EMBO J*. 1992;11(11):3887-3895.
46. Keir ME, Liang SC, Guleria I, et al. Tissue expression of PD-L1 mediates peripheral T cell tolerance. *J Exp Med*. 2006;203(4):883-895.
47. Freeman GJ, Long AJ, Iwai Y, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J Exp Med*. 2000;192(7):1027-1034.
48. Scheffner M, Werness BA, Huibregtse JM, Levine AJ, Howley PM. The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. *Cell*. 1990;63(6):1129-1136.
49. de Jong A, van der Burg SH, Kwappenberg KM, et al. Frequent detection of human papillomavirus 16 E2-specific T-helper immunity in healthy subjects. *Cancer Res*. 2002;62(2):472-479.
50. Welters MJ, de Jong A, van den Eeden SJ, et al. Frequent display of human papillomavirus type 16 E6-specific memory t-Helper cells in the healthy population as witness of previous viral encounter. *Cancer Res*. 2003;63(3):636-641.
51. de Jong A, van Poelgeest MI, van der Hulst JM, et al. Human papillomavirus type 16-positive cervical cancer is associated with impaired CD4+ T-cell immunity against early antigens E2 and E6. *Cancer Res*. 2004;64(15):5449-5455.
52. Eng C, Chang GJ, You YN, et al. The role of systemic chemotherapy and multidisciplinary management in improving the overall survival of patients with metastatic squamous cell carcinoma of the anal canal. *Oncotarget*. 2014;5(22):11133-11142.

53. Ott PA, Piha-Paul SA, Munster P, et al. Safety and antitumor activity of the anti-PD-1 antibody pembrolizumab in patients with recurrent carcinoma of the anal canal. *Ann Oncol*. 2017;28(5):1036-1041.
54. Morris VK, Salem ME, Nimeiri H, et al. Nivolumab for previously treated unresectable metastatic anal cancer (NCI9673): a multicentre, single-arm, phase 2 study. *Lancet Oncol*. 2017;18(4):446-453.
55. Hinrichs CS, Rosenberg SA. Exploiting the curative potential of adoptive T-cell therapy for cancer. *Immunol Rev*. 2014;257(1):56-71.
56. Rosenberg SA, Restifo NP. Adoptive cell transfer as personalized immunotherapy for human cancer. *Science*. 2015;348(6230):62-68.
57. Stevanović S, Draper LM, Langhan MM, et al. Complete regression of metastatic cervical cancer after treatment with human papillomavirus-targeted tumor-infiltrating T cells. *J Clin Oncol*. 2015;33(14):1543-1550.
58. Johnson LA, June CH. Driving gene-engineered T cell immunotherapy of cancer. *Cell Res*. 2017;27(1):38-58.
59. Lamers CHJ, Sleijfer S, Vulto AG, et al. Treatment of metastatic renal cell carcinoma with autologous T-lymphocytes genetically retargeted against carbonic anhydrase IX: first clinical experience. *J Clin Oncol*. 2006;24(13):e20-e22.
60. Parkhurst MR, Yang JC, Langan RC, et al. T cells targeting carcinoembryonic antigen can mediate regression of metastatic colorectal cancer but induce severe transient colitis. *Mol Ther*. 2011;19(3):620-626.
61. Draper LM, Kwong ML, Gros A, et al. Targeting of HPV-16+ epithelial cancer cells by TCR gene engineered T cells directed against E6. *Clin Cancer Res*. 2015;21(19):4431-4439.
62. Hinrichs CS, Doran SL, Stevanovic S, et al. A phase I/II clinical trial of E6 T-cell receptor gene therapy for human papillomavirus (HPV)-associated epithelial cancers [ASCO abstract 3009]. *J Clin Oncol.* 2017;35(15)(suppl).
63. Gross G, Waks T, Eshhar Z. Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. *Proc Natl Acad Sci U S A*. 1989;86(24):10024-10028.
64. DeSelm CJ, Tano ZE, Varghese AM, Adusumilli PS. CAR T-cell therapy for pancreatic cancer. *J Surg Oncol*. 2017;116(1):63-74.
65. Maher J, Brentjens RJ, Gunset G, Rivière I, Sadelain M. Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCR[zeta]/CD28 receptor. *Nat Biotechnol*. 2002;20(1):70-75.
66. Grupp SA, Kalos M, Barrett D, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N Engl J Med*. 2013;368(16):1509-1518.
67. Davila ML, Riviere I, Wang X, et al. Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. *Sci Transl Med*. 2014;6(224):224ra25.
68. Kochenderfer JN, Dudley ME, Kassim SH, et al. Chemotherapy-refractory diffuse large B-cell lymphoma and indolent B-cell malignancies can be effectively treated with autologous T cells expressing an anti-CD19 chimeric antigen receptor. *J Clin Oncol*. 2015;33(6):540-549.
69. Sadelain M. CAR therapy: the CD19 paradigm. *J Clin Invest*. 2015; 125(9):3392-3400.
70. Brown CE, Adusumilli PS. Next frontiers in CAR T-cell therapy. *Mol Ther Oncolytics*. 2016;3:16028.
71. Pule MA, Savoldo B, Myers GD, et al. Virus-specific T cells engineered to coexpress tumor-specific receptors: persistence and antitumor activity in individuals with neuroblastoma. *Nat Med*. 2008;14(11):1264-1270.
72. Louis CU, Savoldo B, Dotti G, et al. Antitumor activity and long-term fate of chimeric antigen receptor-positive T cells in patients with neuroblastoma. *Blood*. 2011;118(23):6050-6056.
73. Basu P, Mehta AO, Jain MM, et al. ADXS11-001 immunotherapy targeting HPV-E7: final results from a phase 2 study in Indian women with recurrent cervical cancer [ASCO abstract 5610]. *J Clin Oncol*. 2014;32(5)(suppl).
74. Cory L, Chu C. ADXS-HPV: a therapeutic Listeria vaccination targeting cervical cancers expressing the HPV E7 antigen. *Hum Vaccin Immunother*. 2014;10(11):3190-3195.
75. Safran H, Leonard KL, DiPetrillo TA, et al. ADXS11-001 Lm-LLO immunotherapy, mitomycin, 5-fluorouracil (5-FU) and intensity-modulated radiation therapy (IMRT) for anal cancer [ASCO abstract e15072]. *J Clin Oncol.* 2017;35(15)(suppl).