Journal of Experimental Pharmacology

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REVIEW

Emerging and Experimental Agents for Anal Cancer: What is New?

This article was published in the following Dove Press journal:
Journal of Experimental Pharmacology

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Abstract: Squamous cell carcinoma of the anal canal (SCCA) is an HPV-related malignancy with rising incidence in the past few decades in the US, characterized by high rates of complete response to chemoradiotherapy with curative intent. However, in a long-term follow-up, a meaningful subgroup of patients with locally advanced disease presents disease recurrence, which demands treatments with high morbidity and important impact in the quality of life. In metastatic or unresectable disease, palliative chemotherapy is the standard of care, but it is still associated with a dismal prognosis. Novel agents are urgently needed in the systemic therapy of SCCA. From a translational standpoint, there are many hurdles to overcome, since *PI3KCA* mutation is the most frequent genetic abnormality and actionable mutations are rarely found in SCCA, as well as it is characterized by low tumor mutational burden and low rates of high-frequency microsatellite instability. But the latest studies of immunotherapeutic approaches have produced promising findings and this therapeutic strategy is the major path being followed in the ongoing clinical trials. The latest advances in the systemic therapy of SCCA have provided the framework for the conception of new clinical trials. Therefore, carboplatin plus paclitaxel have become the backbone for novel agents. Immune checkpoint inhibitors (ICIs), mainly anti-PD-1 monoclonal antibodies, such as retifanlimab, nivolumab, and atezolizumab have been studied in Phase III trials with chemotherapy in first-line therapy. Likewise, ICIs have been evaluated in locally advanced and refractory disease. Novel technologies, such as bispecific antibodies, and immunotherapeutic approaches, such as vaccines and adoptive T-cell therapies, have also been tested in ongoing clinical trials. Immunotherapy may bring practice-changing advances in the systemic therapy of SCCA in the next few years and it might play a larger role in the therapeutic management of this challenging disease.

Keywords: chemotherapy, molecular targeted therapy, immunotherapy, monoclonal antibodies, angiogenesis inhibitors

Introduction

Squamous cell carcinoma of the anal canal (SCCA) is an orphan disease, responsible for approximately 2.5% of all gastrointestinal malignancies.[1] Nevertheless, its incidence rates have been steadily increasing in the past decade, accounting for more than 8500 new cases yearly in the United States.[1–3] Globally, country-specific epidemiological patterns have been observed, but rising incidence rates of the disease have also been described in several high-income countries, such as Australia, Canada, Denmark, France, Italy, Netherlands, and the UK.[4] It is etiologically linked to human papillomavirus (HPV) infection and presents higher incidence rates in immunocompromised patients, especially those infected by HIV.

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DovePress
http://doi.org/10.2147/JEP.S262342

Chemoradiotherapy (CRT) is a highly effective treatment for localized SCCA, associated with complete response (CR) rates in approximately 90% of the patients.[5,6] The combination of infusional 5-fluorouracil (5-FU) or capecitabine with mitomycin concurrent with 50–54Gy of radiation therapy has remained as the standard of care of localized disease since the advent of Nigro regimen.[5–7] However, despite improvements in the radiation therapy techniques, including the incorporation of intensity-modulated radiation therapy (IMRT), it is estimated that 20% to 44% of SCCA patients will present disease recurrence in a long-term follow-up, mainly those with T3/T4 and/or node-positive disease.[5,6,8–11] Disease recurrence is associated with substantial morbidity and mortality. Local recurrence may be managed with salvage surgery, which has a negative impact in the quality of life, and systemic recurrence has a grim prognosis, with an estimated median overall survival (OS) ranging from 12 to 20 months.[12]

Systemic therapy of SCCA is an unmet clinical need. The first randomized clinical trial in advanced disease was published in 2020, and it demonstrated that carboplatin plus paclitaxel should be the standard of care in first-line setting, since it showed superior OS and a more favorable safety profile compared with cisplatin plus 5-FU.[12] The development of clinical trials and targeted therapies in such an orphan disease is challenging. Immunotherapeutic approaches, including the use of immune checkpoint inhibitors (ICIs), has demonstrated promising results in refractory metastatic disease,[13,14] but new therapeutic strategies are urgently needed in the management of patients with recurrent and/or metastatic disease.

In this review, we intend to present the main strategies being explored in the therapeutic development of the systemic therapy of SCCA, the ongoing clinical trials, and the perspectives for the management of this complex disease.

Standard of Care

CRT has remained as the standard of care of locally advanced SCCA for nearly five decades.[7] The Nigro regimen is composed of infusional 5-FU 1000 mg/m^2 on days 1 to 4 and 29 to 32, plus mitomycin 10 mg/m^2 on day 1, concurrent with 50–54Gy of radiation therapy (RT).[7] T1N0 tumors with well or moderately differentiated histology may be considered to excision alone, since there is adequate margins and sphincter preservation.[15,16] NCCN Guidelines suggest that local excision upfront should be reserved to superficially invasive anal cancers, defined as completely excised lesion with less than 4mm of basement membrane invasion and a maximal horizontal spread of less than 8mm.[15]

5-FU plus mitomycin is the regimen of choice with concurrent RT, but the combination of 5-FU plus cisplatin has demonstrated comparable efficacy.[6,11] Based on the phase III RTOG 98–11 trial, 5-FU plus mitomycin was associated with significantly higher disease-free survival (DFS) (68% versus 58%) and OS (78% versus 71%) compared with 5-FU plus cisplatin.[6,11] On the other hand, another phase III trial, ACT II, showed no statistically significant difference between the two regimens in CR rates at 6 months (90.5% vs 89.6% with mitomycin versus cisplatin, respectively), colostomy-free survival (CFS), DFS, and OS.[5] There was no statistical difference in the toxicities of the two regimens, with the exception of higher hematological toxicity in the mitomycin arm.

ACT II trial also demonstrated that the timing of achievement of complete clinical response is later than the historically recommended 6–8 weeks. The decision to perform abdominoperineal resection as surgical salvage for persistent disease should not be made before 26 weeks, given the typical slow regression of the SCCA following RT and the ability to still achieve a complete response.[5] A biopsy of the anal canal for assessment of complete response is discouraged due to the high risk of ulceration and risk of poor wound healing following completion of RT.

For patients with metastatic disease, systemic chemotherapy remains the standard treatment. Based on the randomized Phase II InterAACT trial, carboplatin plus paclitaxel present higher OS (20.0 versus 12.3 months, p=0.014) and lower toxicity rates compared to 5-FU plus cisplatin. Recent single-arm phase II study also suggests high therapeutic activity (89% of objective response) of the triplet regimen DCF (docetaxel plus cisplatin plus 5-FU) in first-line therapy of metastatic SCCA. However, owing to the high toxicity rates, this combination should be reserved to fit patients who need more intensive therapy.[17]

There is no standard therapy for patients who fail to first-line systemic therapy. If not previously used, the combination of 5-FU plus cisplatin may be considered. Recent single-arm phase II trials demonstrated promising findings of ICIs in refractory patients. Nivolumab reached

an overall response rate (ORR) of 24% and pembrolizumab 12%.[13,14]

Molecular Characterization of SCCA

Some of the molecular abnormalities associated with the malignant transformation triggered by HPV infection in epithelial cells are common to all HPV-related malignancies, irrespective of the primary site, but cervical cancer, HPV-positive head and neck cancer, and SCCA have specific genomic and epigenomic alterations that must be unveiled for a more rationale therapeutic development in these tumors.

The description of the tumor microenvironment in SCCA, such as PD-L1 expression, as well as the identification of potential prognostic and predictive biomarkers, and the characterization of the epigenomic, genomic and transcriptomic abnormalities are paramount for the conception of clinical trials addressing novel therapies, such as immunotherapy and targeted therapy.[18]

The completion of comprehensive genomic profiling studies in rare tumors is arduous, but recent studies have provided pivotal data. PIK3CA gene mutation is the most frequent genetic abnormality in SCCA, identified in 32% to 88% of the tumors.[19–22] Genes important in histone modification, such as *MLL3* and *MLL2*, were also found frequently mutated, as well as genes important to DNA damage repair (*p53*, *ATM*, *BRCA 1*, *BRCA 2*), chromatin remodeling (*EP300*, *SMARCB1*, *SMARCA4*), and activation of Wnt/β-catenin signaling (*FAM123B*).[19]

It is also suggested that SCCA has a low tumor mutational burden (TMB), with a mean number of 2.5–3.5 somatic mutations/Mb, similar to those identified in other HPV-related malignancies.[20] It seems that TMB is low even in the uncommon HPV-negative SCCA, which is associated with a higher probability of p53 mutation.[20]

Interestingly, clinically relevant genomic alterations such as KRAS, NRAS, BRAF, EGFR and HER2 are rare in SCCA.[19,20,22] Additionally, DNA methylation status seems to differ according to tumor volume, which raises the hypothesis of a potential role of epigenetic events in the progression of the disease.[18,21]

Therapeutic Agents

Immunotherapeutic Approaches

Immune Checkpoint Inhibitors (ICIs)

HPV infection is associated with a pro-tumorigenic and immunosuppressive local microenvironment, which also presents accumulation of PD-L1-expressing M2 macrophages, contributing to the suppression of cytotoxic T-cell responses.[19,20,23–26] Nevertheless, the efficacy of immunotherapy, mainly of ICIs, has been modest in an unselected population of patients with SCCA. The reasons to explain the moderate efficacy are not clear, but they may be associated to low TMB and to low rates of high-frequency microsatellite instability (MSI-H) found in anal tumors, as aforementioned. Correlative studies from Epitopes-HPV01 and 02 trials have demonstrated that SCCA patients exposed to regimen composed of docetaxel, cisplatin, and 5-fluorouracil (DCF) showed enhanced anti-telomerase immunity, and that high levels of monocytic-myeloid-derived-suppressor cells (MDSC) were associated with poorer prognosis.[27] Anti-telomerase CD4+ Th1-immunity and MDSC may also be implicated in the sensitivity to ICIs. Nevertheless, a small subgroup of SCCA patients seems to be especially sensitive to immunotherapeutic approaches.

The first study to explore such potential was the phase II NCI9673, which evaluated 37 refractory patients with advanced SCCA, irrespective of PD-L1 expression.[13] With a median follow-up of 10.1 months, the use of nivolumab 3mg/kg each two weeks was associated with an overall response rate (ORR) of 24%. Two patients reached CR, with duration of response longer than 24 months in one of them. In addition, seventeen patients showed stable disease, with an overall disease control rate (DCR) of 72%, and a median duration of treatment of 5.8 months. Anemia, fatigue, rash, and hypothyroidism were the grade 3 adverse events described, with a rate of 14%. Correlative studies demonstrated that responder patients had higher baseline percentages of CD8+ T cells and Granzyme B. Among the patients with CD8+ T cells, responders also had higher expression of PD-1, PD-L1, LAG-3, and TIM-3, compared with the non-responders.

Phase Ib KEYNOTE-028 trial had 20 different cohorts of previously treated patients with PD-L1-positive advanced tumors. In the anal cancer cohort, 32 (74%) out of 43 screened patients presented PD-L1 expression ≥1% in the tumor cells.[28] Twenty-five patients, of which 52% had been submitted to two previous lines of therapy, were submitted to pembrolizumab 10mg/kg each two weeks. ORR was 17%, and 10 patients showed stable disease, leading to a DCR of 58%. Median PFS and OS were 3.0 months and 9.3 months, respectively. The use of pembrolizumab in SCCA patients was safe, with no unexpected toxicities.

The phase II study KEYNOTE-158 analyzed the use of pembrolizumab in 112 previously treated patients with SCCA, irrespective of PD-L1 expression.[14] In the overall population, 67% had PD-L1 expression ≥1% and 75% of the patients had been submitted to at least two lines of systemic therapy. ORR was 11.6%, of which 6 patients reached CR. PD-L1-positive patients presented higher ORR compared to the negative counterparts: 14.7% versus 3.3%, respectively. The median PFS was 2.0 months and the OS was 11.9 months.

The new humanized anti-PD-1 monoclonal antibody retifanlimab (INCMGA0012) was evaluated in the Phase 2 study POD1UM-202, with 94 patients with advanced SCCA after platinum failure, regardless of PD-L1 status.[29] Interestingly, 10% of the overall population was composed of HIV-positive patients, since they had negative viral load, CD4 cells ≥300/mm3, and were on antiretroviral therapy. The ORR was 13.8%, and there was no difference based on HIV or PD-L1 status. The median duration of response (DOR) was 9.5 months. The median PFS and OS were 2.3 months and 10.1 months, respectively. Despite the presence of HIV-positive patients, the safety profile was similar to that found in HIV-negative patients and to the other anti-PD-1 monoclonal antibodies.

Likewise, the safety of ICIs in the population of patients with SCCA and HIV infection was also demonstrated in the AMC 095 study.[30] Nivolumab was used in 37 HIV-positive patients with advanced solid tumors and CD4 count >100 cells/μL. Five patients had SCCA. ORR was 24% and the rate of serious treatment-related adverse events (TRAEs) was 11%. The viral load remained stable and negative in 97% of the patients throughout the treatment. On the other hand, there was a trend for a reduction in the CD4 count at 6-week interval.

Adoptive T-Cell Therapies

Adoptive T-cell therapy involving the modification of T-cell receptors (TCR) and the creation of chimeric antigen receptors (CAR) has demonstrated promising findings in SCCA. In a phase I/II study with 9 patients with advanced HPV-positive tumors, of which 4 had SCCA, patients had their T-cells extracted and modified to identify the HLA-A 2:1 epitope HPV16 E6 (E6 TCR T-cells) followed by an autologous infusion.[31] Two partial responses were obtained, lasting 3 and 6 months. No dose-limiting or autoimmune effects were observed.

Likewise, preliminary results of the Phase I study with modified T-cells were recently presented to identify the HLA-A 02:01 HPV16 E7 epitope (E7 TCR T-cells) in patients with refractory HPV16-positive tumors.[32] In the cohort of 12 patients, there were 2 patients with previously treated advanced SCCA. Both patients experienced partial responses lasting 3 and 9 months.

Other immunotherapeutic agents have also been evaluated in advanced SCCA. Axalimogene filolisbac (ADXS11-011) consists of a live, irreversibly attenuated and nonpathogenic strain of the intracellular bacterium *Listeria monocytogenes*, which is bioengineered to secrete an antigen-adjuvant fusion protein between Listeriolysin O (LLO) and the HPV-16 E7 oncoprotein.[33] It may stimulate tumor-specific responses against HPV-associated cancers. In a first-in-human phase 2 study of 36 previously treated patients with advanced SCCA, ADXS11-011 induced an ORR of 3.4% and a median 6-month PFS of 15.5%.[33] In addition, grade 3 adverse events were noted in 10 patients, with the majority being cytokine-release symptoms. One grade 4 adverse event was noted, and no grade 5 adverse events occurred. Despite being safe, the primary endpoints (ORR ≥ 10% or 6-month PFS rate ≥20%) were not met to proceed to the second phase of the study.

The hypothesis that anti-PD1 therapy may augment vaccine-induced immune responses was tested in a phase II trial of the combination of ISA 101, a synthetic long-peptide HPV-16 vaccine, with nivolumab in patients with incurable HPV16-positive cancers.[34] There was 1 patient with SCCA in the cohort of 24 patients. ORR was 33%, median PFS was 2.7 months, and median OS was not achieved in a median follow-up of 8.6 months. The treatment was safe, with one patient each presenting grade 3 transaminase and grade 4 lipase elevations. Such promising findings deserve further evaluation in a larger randomized clinical trial.

Anti-EGFR Monoclonal Antibodies

Since *RAS* and *BRAF* mutations are rare in SCCA and epidermal growth factor receptor (EGFR) is commonly overexpressed in squamous tumors, anti-EGFR therapy has been intensively explored in the treatment of SCCA. The use of cetuximab and panitumumab has been described in retrospective series, either as single-agent or in combination with chemotherapy, with promising results.[35] Study with 56 patients with previously treated advanced SCCA reported ORR of 41%, median PFS of 4.3 months, and OS of 16.0 months.[36] Cetuximab and panitumumab were used by 63% and 37% of the

patients, respectively. In 90% of the patients, the monoclonal antibodies were used in combination with chemotherapy. Grade 3 and 4 adverse events were not reported.

The recently presented randomized phase II study CARACAS addressed the safety and efficacy of the dual PD-L1 and EGFR blockade.[37] Sixty patients with refractory SCCA were randomized to avelumab alone or in combination with cetuximab. Inclusion of HIV-positive patients were allowed since they were on antiretroviral therapy and had negative viral load. ORR, the primary endpoint, was 17% in the combination arm versus 10% in the avelumab alone arm. With a median follow-up of 11 months, median PFS was 3.88 months versus 2.05 months, respectively. The most common TRAEs were fatigue in 17% of the patients in avelumab arm, and skin and subcutaneous disorders in the combination arm (87%), but only 2 patients (7%) permanently interrupted the treatment due to TRAE.

Antiangiogenics

Angiogenesis mediated by vascular endothelial growth factor (VEGF) is associated with immune evasion mechanisms and an immunosuppressive tumor microenvironment. The combination of immunotherapy with antiangiogenics has been successful in the treatment of hepatocellular carcinoma, and it was recently evaluated in a phase II study with 20 previously treated patients with advanced SCCA.[38] The combination of atezolizumab and bevacizumab reached an ORR of 11%, with 11 additional patients presenting stable disease. With a median follow-up of 9.6 months, median PFS and OS were 4.1 and 11.6 months, respectively. However, the rate of grade 3 and 4 adverse events was 35%, and 1 patient died due to intestinal perforation.

Others (Miscellanea)

Other strategies have been explored, such as PEN-866, which is a miniature drug conjugate that links a heat shock protein 90 (HSP90) binding small molecule to a SN-38 cytotoxic payload. HSP90 is highly expressed in advanced malignancies.[39] PEN-866 targets and binds to activated tumor HSP90 protein, releases its cytotoxic payload, and may result in tumor regression. The first-in-human phase I study evaluated 30 patients with advanced refractory solid malignancies. The only one responder was a patient with SCCA, but six additional patients presented decrease of the target lesions. The most frequent (≥20%) adverse events were nausea (50%), fatigue (43%), and diarrhea (40%).[39]

Perspectives

The latest advances in the systemic therapy of SCCA have provided the framework for the conception of new clinical trials (Table 1). Therefore, carboplatin plus paclitaxel and mDCF have become the backbone for novel agents. Promising findings derived from the studies with ICIs in refractory disease have prompted their evaluation in first-line setting and in locally advanced disease. Anti-PD-1 monoclonal antibodies, such as retifanlimab and nivolumab, have been studied in phase III trials evaluating the combination with carboplatin plus paclitaxel in first-line therapy. Atezolizumab is also being studied in combination with the triplet mDCF in advanced disease. Ongoing randomized clinical trials are investigating the use of ICIs in locally advanced disease, either in combination with chemotherapy or as consolidation therapy after chemoradiation. In refractory disease, the combination of ipilimumab plus nivolumab has also been explored in a randomized phase II study. Novel technologies, such as XmAb20717, a bispecific antibody that simultaneously targets the immune checkpoint receptors PD-1 and CTLA-4, are being investigated in phase I studies with SCCA patients, as well as other immunotherapeutic approaches (Table 1). Based on the ongoing clinical trials evaluating new agents in SCCA, it is possible that immunotherapy begins to play a larger role in the therapeutic management of this challenging disease in the next few years.

Conclusions

SCCA is an HPV-related malignancy with rising incidence in the past few years, characterized by high rates of complete response to chemoradiotherapy. However, a meaningful subgroup of patients with locally advanced disease presents disease recurrence in long-term follow-up, demanding treatments with high morbidity and important impact in the quality of life. In metastatic or unresectable disease, palliative chemotherapy may be used, but it is still associated with a dismal prognosis. Novel agents are urgently needed in the systemic therapy of SCCA. Immunotherapeutic approaches are the main therapeutic strategy being investigated in anal cancer, either with immune checkpoint inhibitors or with vaccines and adoptive T-cell therapies. Combination of immunotherapy with anti-EGFR monoclonal antibodies

Table 1 Ongoing Clinical Trials Evaluating Systemic Therapy in Anal Cancer*

Population	Intervention	Control Arm	Other Tumors Included	ClinicalTrials.gov Identifier
Phase III				
Locally advanced or Metastatic	Carboplatin + Paclitaxel + Retifanlimab	Carboplatin + Paclitaxel + Placebo	No	NCT04472429
Metastatic	Carboplatin + Paclitaxel + Nivolumab	Carboplatin plus Paclitaxel	No	NCT04444921
Advanced	Anti-PD-1/PD-L1 1 year	Anti-PD-1/PD-L1 until disease progression	Yes	NCT04157985
High risk stage II–IIIB (after treatment)	Nivolumab	No	No	NCT03233711
Randomized phase II				
Locally advanced	Durvalumab + Mitomycin + 5FU with RT	Mitomycin + 5FU with RT	No	NCT04230759
Metastatic	Ipilimumab + Nivolumab	Nivolumab	No	NCT02314169
Metastatic or unresectable	mDCF + Atezolizumab	mDCF	No	NCT03519295
Single-arm phase II				
Advanced	Avelumab + Valproic acid	-	Yes	NCT03357757
Metastatic	Pembrolizumab	-	No	NCT02919969
Metastatic or unresectable	Pembrolizumab	-	Yes	NCT02628067
Phase I				
Metastatic/recurrent	PDS0101 + M7824 + NHS-IL12	-	Yes	NCT04287868
Advanced	XmAb20717	-	Yes	NCT03517488
Advanced	Ipilimumab + Nivolumab	-	Yes	NCT02408861

Note: *Active and recruiting on November 28, 2020.
Abbreviations: PD-1, programmed cell death-1; PD-L1, programmed cell death-1-ligand; 5FU, 5-fluorouracil; RT, radiation therapy; mDCF, modified docetaxel, cisplatin, 5-fluorouracil.

or with antiangiogenics have also been evaluated in clinical trials, which may bring practice-changing advances in the systemic therapy of SCCA in the next few years.

Disclosure

The authors report no conflicts of interest in this work.

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