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REVIEW

Experimental and investigational drugs for the treatment of anal cancer

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ABSTRACT

Introduction: Squamous cell carcinoma of the anal canal (SCCA) is a rare malignancy, but its incidence rates have been increasing in the last decade. Studies have demonstrated that up to 47% of patients with locally advanced disease have high-risk features for treatment failure. The potential high rates of recurrence after standard chemoradiotherapy for locally advanced disease and the lack of established care for metastatic disease have created an urgent need for the evaluation of new drugs that will ultimately improve the efficacy of treatment.

Areas covered: This review presents results of recent phase-I and -II clinical trials which evaluate novel therapeutic modalities. The review also describes the findings of comprehensive genomic profiling studies which provide insights for promising therapeutics.

Expert opinion: HPV vaccination is underutilized in the United States and as a result, HPV-associated malignancies are likely to continue for several decades; however, pivotal breakthroughs may create a foundation for distinctive treatment approaches for other HPV-associated malignancies for which no other standard of care exists

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1. Introduction

Squamous cell carcinoma of the anal canal (SCCA) is a rare malignancy, responsible for about 2.5% of all gastrointestinal tumors [1]. Despite its rarity, the incidence rates have been increasing in the last decades, with more than 8,500 new cases expected in 2018 in the United States [1]. The majority of SCCA cases are etiologically linked to human papillomavirus (HPV) infection and its incidence is markedly elevated in immunocompromised patients, predominantly those who are HIV-positive.

Combined modality therapy is the cornerstone for treatment of SCCA. External-beam radiation therapy (RT) concurrent with 5-FU and mitomycin-C or cisplatin demonstrate high efficacy, with complete response rates in about 90% of patients [2,3]. However, long-term follow-up shows rates of disease recurrence between 20% and 44%, which is associated with meaningful morbidity and mortality [2–7]. Disease recurrence often demands radical surgery, such as abdominoperineal resection and pelvic exenteration, which negatively impact quality of life. It is estimated that about 20% of patients will die of the disease [2,3,7].

For almost five decades, concurrent chemoradiation therapy remains the foundation of treatment for locally advanced disease, with the inclusion of novel approaches such as intensity-modulated radiation therapy (IMRT), and expansion of drug options in systemic therapy, with recent incorporation of capecitabine [8,9], taxanes [10], and immune checkpoint inhibitors [11,12] in the therapeutic arsenal. As a rare cancer, one of the greatest challenges has been to receive support to conduct clinical trials to evaluate new drugs with the objective of augmenting the efficacy of combined modality therapy and promote prolongation of overall survival and improvement of quality of life of patients with advanced disease.

2. Standard of care of the treatment of SCCA

The majority of patients will present with locally advanced disease where concurrent chemoradiation therapy is provided with curative intent reserving abdominoperineal resection and loss of sphincter preservation for salvage surgery [13]. Historically, the Nigro regimen (5-FU 1000 mg/m^2 per day by continuous infusion days 1 through 4 and 29 through 32, and mitomycin-C 10 mg/m^2 on day 1 only, concurrent with RT with 30 Gy) has remained the standard of care with small variances in mitomycin-C dosing and radiation dose [14], resulting in local failure rates of approximately 35%, 5-year overall survival of 85%, and 5-year colostomy-free survival of 80% [3,7].

Based on the hematological toxicity of mitomycin-C, and the success of cisplatin with concurrent RT in other gastrointestinal malignancies, both drugs were compared in randomized phase-III clinical trials. RTOG 98–11 compared 5-FU plus mitomycin-C plus RT vs. induction 5-FU plus cisplatin followed by concurrent 5-FU plus cisplatin plus RT [5,7]. The primary end point was disease-free survival (DFS). Patients in the 5-FU plus mitomycin-C arm had significantly higher 5-year colostomy-free survival (72% vs. 65%), DFS (68% vs. 58%), and overall survival (78% vs. 71%). However, the final results noted a nonstatistical significance in colostomy-free survival (p = 0.74) [7]. There was no statistical difference in the toxicities of the regimens, with the exception of higher hematological toxicity

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Article Highlights

- Squamous cell carcinoma of the anal canal is a malignancy with rising incidence rates for the past decade.
- Concurrent chemoradiotherapy with curative intent is the cornerstone of the treatment for locally advanced disease. Nevertheless, it is associated with potential high rates of disease recurrence.
- The majority of cases are etiologically linked to HPV infection and its incidence is markedly elevated in immunocompromised patients.
- Comprehensive genomic profiling studies have consistently demonstrated that PIK3CA gene mutation is the most frequent genetic abnormality in the disease.
- EGFR inhibitors have failed in clinical trials owing to their elevated toxicity associated with combined modality therapy.

Novel trials including, molecular targeted therapy, immune checkpoint inhibitors, immunotherapeutic vaccines and adoptive T-cell therapy will be discussed.

This box summarizes key points contained in the article.

in the mitomycin-C arm. The difference in DFS may be potentially attributed to the delay in initiation of chemoradiation therapy on the induction cisplatin arm. The ACT II trial is the largest phase-III trial comparing 5-FU plus cisplatin vs. 5-FU plus mitomycin-C with concurrent RT with a second randomization to maintenance chemotherapy [4]. The primary end point was complete response at 6 months. The investigators noted no differences in efficacy between the two arms. The complete response rate at 6 months was 90.5% vs. 89.6% with mitomycin vs. cisplatin, respectively. Likewise, the colostomy-free survival, DFS, and overall survival were also similar and not statistically different. As in RTOG 98–11, ACT II trial demonstrated increased mitomycin-related hematological toxicity, but similar non-hematological toxicities. There is no evidence favoring the use of induction or consolidation chemotherapy in the systemic therapy of SCCA.

One of the most pivotal findings of the ACT II trial was the timing of achievement of complete clinical response. Historically, it was suggested that the primary tumor should be assessed by clinical exam 6–8 weeks after the completion of chemoradiotherapy with evidence of residual disease. 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 [4]. 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.

In metastatic disease, there is no established standard regimen of treatment. In the first-line setting, regimens containing the combination of 5-FU plus cisplatin are the most accepted based on small retrospective analyses and as recommended by NCCN guidelines [15,16]. Recently, a single-arm phase-II clinical trial demonstrated the efficacy of modified DCF in the treatment of recurrent disease. This regimen should be considered in first-line setting for patients with performance status 0 and 1, given the meaningful toxicity of a triplet regimen [10]. For patients with poor performance status, the use of the combination of 5-FU plus cisplatin may be considered. InterAACT/ECOG EA#2133 is the first randomized phase-II study of 5-FU and cisplatin vs. carboplatin and weekly paclitaxel in surgically unresectable or metastatic anal cancer patients (ClinicalTrials.gov Identifier: NCT02560298) [17]. Though the primary end point of response rate was equivocal at 57%, reduced number of grade 3/4 toxicities were noted in the carboplatin and weekly paclitaxel arm. After a median follow-up of 25.3 months, secondary end points of progression-free survival were in favor of carboplatin and weekly paclitaxel (8.1M vs. 5.7M, $p = 0.375$), but were clearly in favor for overall survival (20M vs. 15.3M, $p = 0.014$). The pivotal results from InterAACT/EA#2133 will likely change the current paradigm of care for treatment-naïve patients from 5-FU and cisplatin to carboplatin and weekly paclitaxel.

In chemorefractory patients, immunotherapy presents as a treatment option. Recent phase-II trials demonstrated activity for both nivolumab and pembrolizumab, with objective response rates of 24% and 17%, respectively [11,12].

3. HPV and carcinogenesis of the SCCA

HPV infection is the most common sexually transmitted disease in the United States. It is a well known relationship between certain HPV types and malignancies, such as those involving the anogenital tract (cervical, anal, vulvar, vaginal, and penile cancers) and head and neck structures. Like in cervical cancer, HPV 16 is the most common type found in anal cancer. The other high-risk types (e.g., HPV 18, 31, 33) can also be associated with anal neoplasm. It seems that the range of types identified depends on the presence of HIV coinfection.

Once infected by a high-risk HPV type, the transformation of a normal epithelial cell of the anal canal into a malignant cell seems to follow similar molecular steps that occur in cervical cancer. HPV genome may be divided into two parts based on the function of the encoded genes: the early (E) region and the late (L) region [18]. Early-region proteins include E6, E7, E1, E2, E4, and E5 and late-region proteins include L1 and L2. E-region proteins interact with vital proteins in the host cell, such as those closely related to cell cycle regulation, cell growth and differentiation, and are the most critical in the pathogenesis of invasive cancer. L-region proteins are responsible for the structure of the viral capsid. The LCR region is located between E- and L-regions, and it does not have gene-encoding regions, but contains promoter and enhancer DNA sequences critical to the regulation of the viral gene transcription [18].

The two viral proteins vital for the HPV oncogenic potential are E6 and E7 [18,19]. They are continuously expressed in anogenital tumors and their activity contributes directly to the maintenance of the malignant phenotype of the host cell. E6 and E7 interact with two well-known proteins in cell homeostasis: p53 and RB, respectively [20–22]. p53 is an important negative regulator of the cell cycle and facilitates nucleic acid repair in case of DNA damage [23]. Thus, the binding and degradation of p53 by E6 protein contributes to cell proliferation and differentiation and to accumulation of cellular chromosomal damage and genome destabilization [20]. The interaction of E6 with p53 may also affect regulation

and/or degradation of the Src family of nonreceptor tyrosine kinases, potentially playing a role in the stimulation of mitotic activity in infected cells [21]. The interaction of E7 with RB protein will have similar consequences. The RB protein inhibits the positive stimulus over the cell cycle and halts cell growth or induces cell apoptosis in response to DNA damage [22]. RB mainly inhibits the E2F transcription factor. Binding to the RB protein, E7 releases E2F factor, which allows for cell cycling with unchecked cell growth in the presence of genomic instability [24,25].

These HPV-induced molecular alterations may result in a premalignant phenotype in the host cell called squamous intraepithelial lesions (SIL), which can be divided in low-grade (LSIL) and high-grade lesions (HSIL) [26]. Such lesions represent a morphologic continuum that can be demonstrated as a spectrum, from lower grade lesions like condyloma and anal intraepithelial neoplasia (AIN) grade 1 to higher grade lesions, such as AIN grades 2 and 3. There is progressive dysplasia along the spectrum, which ends as severe dysplasia and carcinoma *in situ*. Observational studies suggest that invasive cancer originates from HSIL [27,28]. The factors that determine this final transformation are not well known, but it seems that immunosuppression is a contributing factor, since HIV-infected patients and organ transplant patients present higher probability to progress from HSIL to invasive cancer [29].

Over the recent years, it has become evident that the complex relationship between HPV-infected keratinocytes and local immune microenvironment seems to develop a crucial role in the pathogenesis of HPV-related malignancies. At early stages, HPV-infected cells suppress acute inflammation and immune recognition [30,31]. Throughout the progression to invasive cancer, HPV-transformed cells induce to chronic stromal inflammation, mainly mediated by IL-6/JAK/STAT3, which creates a pro-tumorigenic and immunosuppressive microenvironment in carcinogenesis [31]. The recognition of the receptors and metabolic pathways involved in the interplay between HPV-infected cells and immune microenvironment opens possibilities of immunotherapeutic approaches in the therapeutic management of HPV-related malignancies.

4. Molecular characterization of SCCA

The identification of the molecular alterations associated with SCCA is crucial for the development of novel therapeutic strategies. There are genetic alterations common to all HPV-related malignancies, but the description of the molecular portrait specific to anal cancer will provide insights for the design of clinical trials evaluating targeted therapies and genome-guided personalized therapy (Table 1).

At the molecular level, the most prevalent solid tumors were analyzed through broader platforms, including somatic copy number analysis, whole-exome sequencing, DNA methylation profiling, messenger RNA sequencing, microRNA sequencing and reverse-phase protein array, mainly by TCGA (*The Cancer Genome Atlas*) and ICGC (*International Cancer Genome Consortium*). The molecular landscape of SCCA was less extensively detailed, but recent studies have provided more extensive data. Comprehensive genomic profiling studies have consistently demonstrated that PIK3CA gene mutation is the most frequent genetic abnormality in SCCA [32–35]. A study that performed whole exome sequencing, copy number assessment, and gene expression profiling on tumor-normal pairs from 24 patients with metastatic SCCA found that 88% of the tumors had an activating mutation and/or gene amplification of PIK3CA [34]. Another study evaluating tumor samples from 70 patients with SCCA stages II to IV identified PIK3CA mutation in 40% of cases, while a third one identified 32% in a population of 199 patients using next-generation sequencing or Sanger sequencing [32]. MLL3 and MLL2, genes important in histone modification, were also found frequently mutated, as well as genes important to cell cycle dysregulation (CNTRL), DNA damage repair (p53, ATM, HUWE1, BRCA 1, BRCA 2), chromatin remodeling (EP300, SMARCB1, SMARCA4), cell differentiation (FLG, PTK2), and activation of Wnt/β-catenin signaling (FAM123B) [32].

Table 1. Frequency of the most commonly occurring mutations in SCCA.

Gene	Frequency
PIK3CA	29%–40%
MLL3	32%
MLL2	22%
EP300	22%
p53	15%–20%
FBXW7	13%–14%
PTEN	2%–14%
BRCA1	1%–12%
BRCA2	3%–12%
AKT1	3%–7%
EGFR	0%–5%
BRAF	0%–5%
KRAS	0%–4%
NRAS	0%–2%

These studies also suggest 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, such as cervical cancer and HPV-positive head and neck cancer [34]. It seems that tumor mutational burden is low even in the uncommon HPV-negative SCCA, which is associated with a higher probability of p53 mutation [34].

Interestingly, well-known clinically relevant genomic alterations such as KRAS, NRAS, BRAF, EGFR and HER2 are infrequent (< 5%) in SCCA [32,34,35].

Comprehensive genomic profiling did not find association between genomic alterations and disease stage [32]. Nevertheless, it seems that there are different patterns of DNA methylation according to tumor volume. Analysis of DNA methylation status of 121 patients with nonmetastatic SCCA showed that 16 CpG loci were differentially methylated (14 increased and 2 decreased) in locally advanced disease compared to early-stage disease [33]. This finding generates the hypothesis of the potential role of epigenetic events in the progression of the disease.

5. Therapeutic implications

Based on the genomic landscape of SCCA known so far, it is possible to consider therapeutic strategies that act on the affected pathways. In a series composed of tumor samples from 70 patients, at least one clinically relevant genomic alteration was identified in 76% of tumors [32]. Experimental and investigational

drugs such as PIK3CA inhibitors, as well as inhibitors of the EGFR, RAS, mTOR, and immunotherapeutic approaches have been evaluated in preclinical models and clinical trials (Table 2).

5.1. PIK3CA pathway

Phosphatidylinositol 3-kinases (PIK3s) are heterodimeric lipid kinases that consist of several regulatory subunits. In response to stimulation by growth factors, PIK3CA, which encodes the p110alpha catalytic subunit of PIK3, activates downstream effectors, including pAkt and mTOR [36]. PIK3CA amplification contributes to cell proliferation and survival in SCCA carcinogenesis by activating the PIK3/pAkt pathway [37]. PIK3CA mutations have been detected at various frequencies in the overall population, but it is consistently described as the most frequent genetic mutation in SCCA, ranging from 29% to 40% [32,34,35].

Since the hyperactivation of the PIK3CA/Akt/mTOR pathway in SCCA is well known, the role of mTOR inhibition in preclinical experimental models was evaluated. These studies suggest that mTOR inhibitors, such as rapamycin, are active in slowing tumor progression and reducing tumor burden [38,39]. However, there are no clinical trials evaluating these drugs in humans with SCCA.

Table 2. Targeted therapies in SCCA investigated in clinical trials.

Target	Drug	Study phase	Status
PIK3CA	Rigosertib	II	Ongoing (NCT01807546)
EGFR	Cetuximab	I	Available results (NCT01621217)
	Cetuximab	II	Ongoing (NCT00955240)
	Cetuximab	II	Available results (NCT00316888)
	Panitumumab	I/II	Ongoing (NCT01581840)
	Panitumumab	II	Ongoing (NCT01285778)
	Nimotuzumab	II	Available results (NCT01382745)
RAS	Rigosertib	II	Ongoing (NCT01807546)
PD-1/PD-L1	Nivolumab	II	Ongoing (NCT03233711)
	Nivolumab	II	Available results (NCT02314169)
	Nivolumab	II	Ongoing (NCT03233711)
	Pembrolizumab	II	Ongoing (NCT02919969)
	Atezolizumab + Bevacizumab	II	Ongoing (NCT03074513)
	Atezolizumab + mDCF	II	Ongoing (NCT03519295)
CTLA-4	Nivolumab + Ipilimumab	I	Ongoing (NCT02408861)
	Nivolumab + Ipilimumab	II	Ongoing (NCT02314169)
HPV	ADXS11-001	II	Ongoing (NCT02399813)
	ADXS11-001	II	Ongoing (NCT01671488)
HLA-A*02:01-E6	Adoptive T-cell therapy	I/II	Available results (NCT02280811)
MAGEA1-003	Adoptive T-cell therapy	I	Ongoing (NCT03247309)

Despite the strong rationale for the use of PIK3CA inhibitors in SCCA, experimental models with xenograft tumor were exposed to BYL719, a PIK3CA inhibitor, and tumor reduction was not demonstrated compared to controls [34].

Rigosertib, a dual-pathway inhibitor that acts on PIK3CA and polo-like kinase 1 (PLK1) activity, has been tested in malignancies such as pancreatic cancer and myelodysplastic syndromes and is under evaluation in a phase-II study in patients with squamous cell carcinomas [40,41].

Ongoing clinical trials:

- A phase-II study of oral rigosertib in patients with relapsed or metastatic, platinum-resistant, HPV positive or negative squamous cell carcinoma (NCT01807546)

5.2. EGFR pathway

The epidermal growth factor receptors HER1 (also denoted EGFR), HER2, HER3, and HER4 comprise the EGFR family [42,43]. Except for HER3, these receptors all share the same molecular structure: an extracellular domain that binds to the ligand, a transmembrane portion, and an intracellular domain with tyrosine kinase activity [44]. The binding of different ligands to the extracellular domains triggers intracellular signaling reactions involved in cell differentiation, proliferation and survival. The binding of the ligand to the extracellular domain induces the EGFR homodimerization and heterodimerization of the remaining receptors.

Given its origin from squamous epithelium, it was expected that SCCA had a high expression of EGFR, as well as other squamous cell carcinomas. This hypothesis was confirmed by studies which reported EGFR immunoreactivity ranging from 55% to 100% [45]. These studies supported the rationale for the evaluation of anti-EGFR monoclonal antibodies both in localized and metastatic disease.

Small retrospective studies suggest high efficacy with the association of both cetuximab and panitumumab with chemotherapy in recurrent or advanced disease. They reached objective response rates of 30–35% [46,47].

Two phase-I trials evaluated the feasibility and dose-limiting toxicity of the association of cetuximab with chemoradiotherapy for locally advanced disease [48,49]. One study with 23 patients reported 95% of complete response with cetuximab plus 5-FU plus cisplatin concurrent to RT [48]. Another study evaluated chemoradiotherapy composed of cetuximab with 5-FU plus mitomycin for 13 patients (85% with stage IIIB disease) and confirmed the elevated efficacy of the anti-EGFR monoclonal antibody, with 91% complete response rate [49]. Nevertheless, both studies demonstrated substantial rates of grade 3–4 side effects, especially dermatitis (52% to 63%), hematologic (54%), diarrhea (36% to 43%), and thrombosis/embolism (24%).

Three phase-II trials confirmed the expected efficacy and toxicity related to the association of anti-EGFR monoclonal antibodies (cetuximab and panitumumab) concurrent to standard chemoradiotherapy [50–52]. A study with 61 patients treated with platinum-containing regimen, 64% of which had stage III disease, reported 3-year locoregional failure of 23%, progression-free survival of 68% and overall survival of 83%

[50]. However, 32% experienced grade-4 toxicity and 5% of treatment-related deaths, which are unacceptably high rates. Another phase-II study, originally designed to include 81 patients with locally advanced disease, was prematurely stopped after the occurrence of serious adverse events (SAE) in 14 out of 16 patients [51]. Forty-five SCCA patients with HIV-infection received cetuximab concurrent to platinum-containing regimen, and presented 3-year locoregional failure of 42%, progression-free survival of 72% and overall survival of 79% [52]. Likewise non-HIV-infected patients reported elevated toxicity, with 26% having grade-4 adverse events, and 4% treatment-related deaths. Nimotuzumab, another class member of the anti-EGFR monoclonal antibodies, was evaluated concurrent with RT in a phase-II trial, but the study was closed due to low accrual without available results (1 patient enrolled).

Comprehensive genomic profiling studies report low incidence of EGFR mutations in SCCA, ranging from 0% to 5% [32,34,35]. To date, there are no clinical trials evaluating the role of anti-EGFR tyrosine kinase inhibitors in anal cancer.

Ongoing clinical trials:

- Phases I and II on radiochemotherapy combined with panitumumab in the treatment of localized epidermoid carcinoma of the anus (NCT01581840)
- Phase-II nonrandomized multicenter study of the impact of radiochemotherapy (65 Gy + cisplatin + 5-FU) combined with cetuximab in patients presenting with locally advanced anal cancer (NCT00955240)
- Phase-II trial to assess the efficacy and safety of chemoradiation with 5-FU, mitomycin C and panitumumab as a treatment for anal squamous cell carcinoma (NCT01285778)

5.3. RAS pathway

The RAS superfamily is composed by three genes which encode their respective proteins: KRAS, NRAS, and HRAS [53]. Once phosphorylated, these proteins trigger downstream cell signaling, through BRAF and MAPK, which will ultimately lead to cell growth, proliferation and survival. When mutated, they become constitutively activated and cause unintended and overactive signaling inside the cell, even in the absence of incoming signals. RAS mutations, especially KRAS, are one of the most frequent mutations in cancer. Their frequency varies among cancers and can be found in up to 95% of patients, like in pancreatic cancer. Owing to their importance in human cancers and its negative predictive value for the benefit derived from the anti-EGFR monoclonal antibodies in colorectal cancer, they have been studied in SCCA.

More recent comprehensive genomic profiling studies have shown that RAS mutations, as well as BRAF mutations, are rare in SCCA. KRAS, NRAS, and BRAF mutations were found in 4.3, 1.4, and 0%, respectively, in a study with 70 patients using next generation sequencing for the analysis of 236 cancer-related genes [32]. Whole-exome sequencing in a sample of 24 patients found rates of 2, 2, and 5%, respectively [34]. Another study with 199 patients found no KRAS, NRAS and BRAF mutations in a next-generation sequencing analysis [35].

Through activation of the RAS/BRAF/MAPK pathway in SCCA with RAS mutations, it is plausible to evaluate the role of MEK inhibitors, such as cobimetinib and trametinib, in this subgroup of patients. Nevertheless, there are neither preclinical nor clinical studies in SCCA addressing this issue.

Rigosertib, a PIK3CA inhibitor described previously, can also function as multi-target kinase and have inhibitory effects over RAS mutations, has also been tested in patients with squamous cell carcinomas [40,41].

Ongoing clinical trials:

- A phase-II study of oral rigosertib in patients with relapsed or metastatic, platinum-resistant, HPV positive or negative squamous cell carcinoma (NCT01807546)

5.4. Immune checkpoint pathways

It is well known that evasion of the immune system is one of the hallmarks of cancer [54]. At early stages, HPV-infected cells suppress acute inflammation and immune recognition. It was demonstrated that there is suppressed recognition via receptor toll-like receptor 9, as well as inhibition of interferon (IFN) expression, IFN signaling, and downstream responses [55–57]. The HPV oncoprotein E6 inactivates IFN regulatory factor 3 (IRF3), which contribute to immunosuppression in the local microenvironment [58]. Through the progressive stages of infection, HPV-transformed cells suppress IL-6 and activate JAK/STAT3 pathway, which contributes to the formation of a pro-tumorigenic and immunosuppressive microenvironment [59]. Furthermore, owing to the inhibition of IFN production, there is an accumulation of M2 macrophages, which are cells that express PD-L1, contributing to suppression of cytotoxic T-cell responses [60,61]. The better understanding of the relationship between HPV-infected cells and local immune microenvironment underpins the development of immunotherapeutic approaches in the management of SCCA, such as immune checkpoint inhibitors, and other drugs that might target T-cell costimulatory molecules and pathways involved in HPV-related carcinogenesis [31,62].

In recent years, immune checkpoint inhibitors have been evaluated in several tumors, with remarkable results. Monoclonal antibodies targeting CTLA-4 receptor (ipilimumab, tremelimumab), PD-1 receptor (nivolumab, pembrolizumab, durvalumab), and PD-L1 (atezolizumab) have reached revolutionary results particularly in melanoma, renal cell carcinoma, non-small cell lung cancer, and gastrointestinal malignancies with microsatellite instability. However, even in these tumors, the majority of patients submitted to immunotherapy will not benefit from this therapeutic approach. PD-L1 tumor expression has been used as a predictive biomarker of objective response and overall survival, but it seems that TMB could be a better predictive biomarker.

It has been suggested that HPV oncoproteins upregulate immune checkpoint proteins, such as PD-1 [63,64]. Based on this biologic rationale and on the successful experiences in other solid tumors, a phase-II trial was initiated evaluating the role of nivolumab in the treatment of treatment-refractory metastatic SCCA [11]. Thirty-seven patients were enrolled and it resulted in an objective response of 24%. In a small

subset of exploratory correlatives, increased PD-1 expression on CD8 + T-cells, LAG-3, and TIM-3 at baseline was associated with response.

Comprehensive genomic profiling studies consistently show that the vast majority of SCCA patients have low TMB (2.5–3.5 somatic mutations/MB) [34]. One series reports the finding of high TMB in 3 out of 20 patients. Another study compared the mutational analysis pre- and post-chemoradiotherapy in 61 patients, and a meaningful response to anti-PD1 monoclonal antibody in 1 patient, whose genomic analysis revealed high TMB, was observed [65]. Hence, based on current evidence, nivolumab represents an option in the treatment of SCCA patients with treatment-refractory metastatic disease. Rather than PD-L1 expression, TMB analysis could become a useful predictive biomarker in the selection of patients who will derive the greatest benefit from immunotherapy.

Ongoing clinical trials:

- ECOG 2165: A randomized phase-II study of nivolumab after combined modality in high-risk anal cancer (NCT03233711)
- NCI9673 (Part B): A randomized phase-II ETCTN study of nivolumab plus or minus ipilimumab for refractory surgically unresectable or metastatic anal cancer
- Phase-II study of atezolizumab and bevacizumab in rare solid tumors including an HPV-malignancies arm (NCT03074513)
- A multicenter phase-II clinical trial of pembrolizumab in refractory metastatic anal cancer (NCT02919969)
- A phase-I study of ipilimumab and nivolumab in advanced HIV-associated solid tumors with expansion cohorts in HIV-associated solid tumors and a cohort of HIV-associated classical Hodgkin lymphoma (NCT02408861)
- A study of mDCF in combination or not with atezolizumab in advanced squamous cell anal carcinoma (SCARCE) (NCT03519295)

5.5. Immunotherapeutic vaccines

The bacterial vector most commonly used as an immunotherapeutic vaccine base is *Listeria monocytogenes (Lm)*, because of its immunological advantages. Following infection of the host cells, *Lm* has the ability to activate both the innate and adaptive immune responses. *Lm* has been successfully used as a delivery vector for tumor-specific antigens. *Lm*-listeriolysin O (LLO) immunotherapies have been reported to present with multiple simultaneous mechanisms of action that contribute to generation of a therapeutic response.

Axalimogene filolisbac (ADXS11-001; AXAL) is based on the irreversibly attenuated *Lm* fused to the nonhemolytic fragment of LLO, and has been developed to secrete the *Lm*-LLO-E7 fusion protein targeting HPV-positive tumors. It has been widely evaluated in HPV-associated malignancies, primarily in cervical cancer.

AXAL has been investigated in combination with standard of care RT and concurrent 5-FU and mitomycin-C in a phase-I study in patients with high-risk locally advanced SCCA (BrUOG 276). Nine out of 11 patients had complete remission with a well-tolerated safety profile [66]. Phase-II trial evaluated the efficacy and safety of AXAL in patients with surgically unresectable or metastatic SCCA [67]. In a first stage of the study, it demonstrated promising activity with 1 partial remission in 29 patients and was just shy of reaching its primary end point, 6-month PFS, at 19%. Additional development has ceased with final results to be reported. A Phase 2, Open-Label Study to Evaluate Efficacy of Combination Treatment with MEDI0457 (INO-3112) and Durvalumab (MEDI4736) in Patients with Recurrent/Metastatic Human Papilloma Virus Associated Cancers is due to open shortly (NCT03439085). MEDI0457 is an investigational T-cell activation immunotherapy that targets cancers caused by HPV types 16 and 18.

5.6. Adoptive T-cell therapy

Preclinical experiments evaluating chimeric antigen receptors (CARs)-expressing T cells as cancer therapy were initiated in 1993 [68,69], and this technique has been assessed in several tumors, mainly B-cell malignancies [70,71]. It consists of a procedure in which peripheral T lymphocytes are extracted from the patient, followed by their preparation for adoptive transfer by genetically modifying the T cells to express receptors that specifically recognize tumor-associated antigens [72]. There are two methods for generating antigen-specific T cells by genetic modification: introducing genes encoding αβ T-cell receptors (TCRs) or introducing genes encoding CARs [72–74]. TCRs recognize peptides presented by human leukocyte antigen (HLA) molecules. Therefore, a particular TCR must be used only in patients expressing certain HLA molecules. On the other hand, CARs recognize intact cell-surface proteins and glycolipids, which becomes CARs independent from HLA type [72–75].

Phase I/II clinical trial of T cells genetically engineered to express a TCR that targets an HLA-A*02:01-restricted epitope of E6 (E6 TCR T Cells) for patients with metastatic HPV-16 + carcinoma evaluated 16 patients, of which 4 were anal cancer patients [76]. Two patients with anal cancer showed partial responses lasting 3 and 6 months after treatment. The patient with a 6-month response had complete regression of one tumor and partial regression of two tumors that were resected upon progression.

Ongoing clinical trials:

- TCR-engineered T Cells in Solid Tumors With Emphasis on NSCLC and HNSCC (ACTengine) (NCT03247309)

6. Conclusion

SCCA is a rare malignancy of the gastrointestinal tract with a rising incidence in the last decade. The development of SCCA has been closely linked to prior HPV exposure. Immunosuppression is a clear risk factor for the progression of premalignant lesions of the anal canal to invasive carcinomas. Despite the high efficacy of combined modality therapy, approximately 40% of patients will present with disease relapse, and about 20% of all patients will die of the disease [2,3,7]. Carboplatin and weekly paclitaxel will likely be considered a new standard of care for treatment-naïve metastatic

anal cancer patients following the pivotal results of the randomized phase-II InterAACT/EA#2133 with additional studies pending or ongoing in the refractory setting.

Cancer therapy has been changing dramatically in the last years from the recognition of the complex interplay between specific genetic and epigenetic events, which provide insights for the development of targeted therapies and genome-guided personalized therapy. It has consistently demonstrated that PIK3CA mutations are the most frequent genetic abnormalities in SCCA, and therapeutic approaches can be developed to target this pathway. Genomic studies have also found that SCCA has a very low TMB in contrast to other HPV-associated malignancies. Therefore, other predictive factors need to be identified.

Phase-I and -II trials evaluating the role of anti-EGFR monoclonal antibodies were unsuccessful owing to their elevated toxicity associated with chemotherapy and concurrent to RT. Interestingly, well-known targetable mutations, such as RAS, BRAF, EGFR and HER2 are infrequent in SCCA and it is not justifiable to incorporate these tests in the management of the disease.

From recent comprehensive genomic profiling studies, it is possible to recognize the genomic heterogeneity of SCCA, which will permit the development of clinical trials with targeted therapies. Based on these molecular findings and through development of new studies with broader platforms, such as DNA methylation profiling and microRNA sequencing, added to those already performed, it will provide a roadmap to implement precision medicine in SCCA.

7. Expert opinion

SCCA continues to remain a 'rare cancer' in the United States with <10,000 individuals diagnosed per year: globally, 35,000 individuals per year [77]. With most patients presenting with locally advanced disease and the success of combined chemoradiation therapy with curative intent, the treatment of metastatic disease was not recognized as an unmet need. Yet, for the past decade, the incidence of anal cancer continues to rise 2.2% per year [78]. In the United States, 2017 resulted in the highest incidence of sexually transmitted diseases (chlamydia, gonorrhea, and syphilis combined) for the fourth consecutive year, affecting 2.3 million. In 2013, a CDC analysis estimated 20 million new U.S. STD's (including HPV), resulted in an astounding $16 billion in medical costs [79]. Despite these findings, HPV vaccination continues to remain underutilized in the United States. It is estimated that 66% adolescents aged 13–17 years received the first dose but only 49% of adolescents received all the recommended doses to complete the series [80]. Thus, the incidence of HPV-related malignancies will continue to rise over the next several decades.

Though immune checkpoint inhibition appears to be a highly promising treatment option based on two single-agent, single-arm, phase-Ib and -II studies, only a small minority of patients will benefit (RR = 17%–24%). Thus far, predictive markers for benefit have not yet been identified. In the single-arm NCI9673 (Part A) trial, one patient remains in complete response (CR) for >2 years. As in other cancers responsive to immunotherapy, the optimal duration of therapy remains unknown. Ongoing studies are exploring the role of immune checkpoint inhibition in high-risk locally advanced patients following chemoXRT and in the refractory metastatic patient population as a single agent and in combination with the CTLA-4 inhibitor, ipilumumab, respectively; tissue and blood correlates will hopefully designate predictive markers of benefit. However, the risk of doublet immunomodulation may place the patient at risk for potentially greater toxicities at the cost of efficacy of therapy. The final results of NCI9673 (Part B) will hopefully provide these answers.

We are finally seeing a series of clincial trials for the rare malignancy of SCCA for both early and late stage disease. Additional questions remain unanswered including the role of immune checkpoint inhibition in the metastatic treatment-naive patient population as well as the role of immune checkpoint inhibition with RT (whether it is as a radiation sensitizer or for an instigator of the enigmatic abscopal effect). Alternate treatment options must continue to be explored for patients who fail to respond or progress to immune checkpoint inhibition whether it is combined with other immunomodulatory agents such as LAG-3 or TIM-3, oncolytic viruses, or with other bioengineered vaccines. In conclusion, though considered a rare malignancy, it should be acknowledged that pivotal breakthroughs made in SCCA may help create a foundation for distinctive treatment approaches for other HPV-associated malignacies for which no other standard of care exists.

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