Cancer Treatment Reviews 105 (2022) 102373

Contents lists available at ScienceDirect

Cancer Treatment Reviews

journal homepage: www.elsevier.com/locate/ctrv

Anti-tumour Treatment

Immunotherapy in advanced anal cancer: Is the beginning of a new era?

Davide Ciardiello [a,b,*,1], Luigi Pio Guerrera [a,b,1], Brigida Anna Maiorano [a,c,1], Paola Parente [d], Tiziana Pia Latiano [a], Massimo Di Maio [e], Fortunato Ciardiello [b], Teresa Troiani [b], Erika Martinelli [b], Evaristo Maiello [a]

[a] Oncology Unit, Casa Sollievo della Sofferenza Hospital, 71013 San Giovanni Rotondo, FG, Italy
[b] Oncology Unit, Department of Precision Medicine, Università degli Studi della Campania "Luigi Vanvitelli", 80131 Naples, Italy
[c] Department of Translational Medicine and Surgery, Catholic University of the Sacred Heart, 000168 Rome, Italy
[d] Pathology Unit, Fondazione IRCCS Ospedale Casa Sollievo della Sofferenza, 71013 San Giovanni Rotondo, FG, Italy
[e] Department of Oncology, Università degli Studi di Torino, at Azienda Ospedaliera Ordine Mauriziano di Torino, Turin, Italy

ARTICLE INFO

Keywords:
SCCA
Immunotherapy
ICIs
Biomarkers
HPV-related tumors

ABSTRACT

For decades metastatic squamous cell carcinoma of the anus (SCCA)has been considered a rare disease with very limited treatment options and a dismal prognosis. Prior to 2017, no data from prospective studies on the management of metastatic SCCA were available with scant information from retrospective analyses and few treatment options. Recently, InterAAct trial showed an advantage of carboplatin plus paclitaxel over the historical standard of care represented by cisplatin plus 5-fluorouracil. Unfortunately, there is no established second-line treatment after progression to first-line platinum-based chemotherapy. Interestingly, a better understanding of the immunobiology of the neoplasm and the strict association between HPV/HIV infection and tumor microenvironment led to the development of immunotherapies. Emerging evidence suggests that the use of anti-PD1/PD-L1 agents could lead to promising antitumor activity in a subgroup of patients with pre-treated anal cancer, opening new therapeutic scenarios. Here, we will focus on completed clinical trials evaluating immunotherapy in patients with (SCCA), pointing out the future perspectives and possible biomarkers of response.

Introduction

Anal cancer is a rare disease that accounts for<3% of all gastrointestinal tumors [1]. The incidence of its most common histologic variant, squamous cell carcinoma of the anus (SCCA) is 0.5–2 cases/100.000 per year, although it has been steadily increasing in the latest years [1-5].

At diagnosis, approximately 80–90% of patients present with localized disease and undergo definitive curative intent treatment [1].

The pathogenesis of anal cancer is very complex and still a topic of debate.

Human Papilloma Virus (HPV) infection has demonstrated a causative role for 80–90% of SCCA [1,4,6]. However, the infection alone is not enough for tumorigenesis, and additional factors are involved, including a high number of sexual partners, human immunodeficiency virus (HIV) infection or other forms of immune suppression, history of other HPV-related cancers and cigarette smoking [1,2,6,8-10].

For decades anal cancer has been considered an orphan disease with very limited treatment options [1,2,7]. As to localized SCCA, definitive curative intent chemo-radiotherapy (CRT) regimen based on mitomycin C (MMC) and 5-fluorouracil (5-FU) with concomitant radiotherapy (RT) has remained the standard of care, achieving high rates of complete responses (80–90%), with surgery simply reserved as a salvage option for non-responders or for locally recurrent disease [1,2,4,6].

However, approximately 10–20% of patients suffer from distant recurrence. Moreover, in about 10% of cases, patients present with metastases at the time of diagnosis. Differently from localized disease, the prognosis of patients with advanced anal cancer is poor with a 5-year relative survival rate lower than 30% [1,4,6,7].

Prior to 2017, no data from prospective studies on the management of metastatic SCCA were available, with scant information from retrospective analyses and few treatment options [3,4].

The historically recommended treatment in the first-line setting was

* Corresponding author.
E-mail address: davide.ciardiello@unicampania.it (D. Ciardiello).
[1] Contributed equally.

https://doi.org/10.1016/j.ctrv.2022.102373
Received 11 February 2022; Received in revised form 27 February 2022; Accepted 28 February 2022
Available online 8 March 2022

the combination of cisplatin + 5FU [1,6]. However, in the last years, the scenario is gradually changing. On the basis of the encouraging preliminary results of the Epitopes-HPV01 trial, the single-arm, phase II, Epitopes-HPV02 study evaluated the combination of docetaxel, cisplatin and 5-FU (DCF) for 6 cycles or 8 cycles of modified DCF (mDCF) showing a progression free survival (PFS) at 1 year and an overall response rate (ORR) of 47% and 89% respectively [11-13]. A pooled analysis of updated data from both the studies confirmed the encouraging results in terms of ORR (87.7%), median PFS (mPFS)(12.2 months) and median overall survival (mOS, 39.2 months) as well as the absence of differences among standard DCF and mDCF in terms of OS and PFS [7,14].

The InterAAct study was the first, prospective, randomized, phase II trial that evaluated the combination of carboplatin + paclitaxel (experimental treatment) compared with cisplatin + 5FU (control arm), as first-line treatment [15]. Although there was no statistically significant difference in terms of ORR (59% vs 57%) and PFS among the two arms, an improvement in median OS (20 vs 12.3 months) was described in the experimental arm [1,4,15]. However, this finding must be interpreted cautiously given the small number of patients enrolled.

Based on these results and the better safety profile, carboplatin + paclitaxel regimen should be considered as a new standard of care in CT-naïve metastatic/advanced SCCA patients [1,6]. In the second-line setting, cisplatin, 5-Fluorouracil, taxanes, doxorubicin, irinotecan +/- cetuximab, and mitomycin plus 5-Fluorouracil can be all considered alone or in combination, but evidence-based robust data are still lacking [1,6].

Interestingly, results of a second-line *post hoc* study of the Epitopes-HPV01 and Epitopes-HPV02 trials were recently published [14,18]. No significant differences were observed between regimens; furthermore, reintroduction appeared as a feasible option in responders. [16]

In this scenario, the use of immune checkpoint inhibitors (ICIs) demonstrated promising evidence of clinical activity in a subgroup of patients with pre-treated SCCA [17,18]. Subsequently, several immunotherapeutic approaches including ICIs, vaccines, adoptive T cell therapy, are gaining ground in this orphan scenario and are being tested alone or in combination in second line and even in other settings [19].

Herein we will focus on the studies that were completed and are still ongoing for metastatic disease, pointing out the future perspectives and possible biomarkers of response.

Immunobiology of anal cancer

HPV infection as a driver of tumor initiation, progression and immune evasion

The antitumor activity of immunotherapy reported in metastatic SCCA is the result of a particular tumor biology and microenvironment, which render most of these tumors particularly immunogenic, although they rarely harbor mismatch repair deficiency (dMMR) and usually show a low tumor mutational burden (2,5–3,5 somatic mutations/megabase) [9,19-21].

Immunogenicity is closely related to HPV infection, which is recognized as the causative agent of the vast majority of SCCA (80–90% of cases) (Fig. 1). HPV16 is the most frequently identified genotype (80% of cases), followed by HPV 33, HPV 18 and HPV 58 [4].

The viral genome can be either maintained as extra chromosomal episomes or integrated into the host DNA [9]. Integration is undoubtedly one of the mechanisms by which HPV evades immune response, along with its ability to induce programmed death ligand 1 (PD-L1)-overexpression and transforming growth factor beta (TGFβ) pathway up-

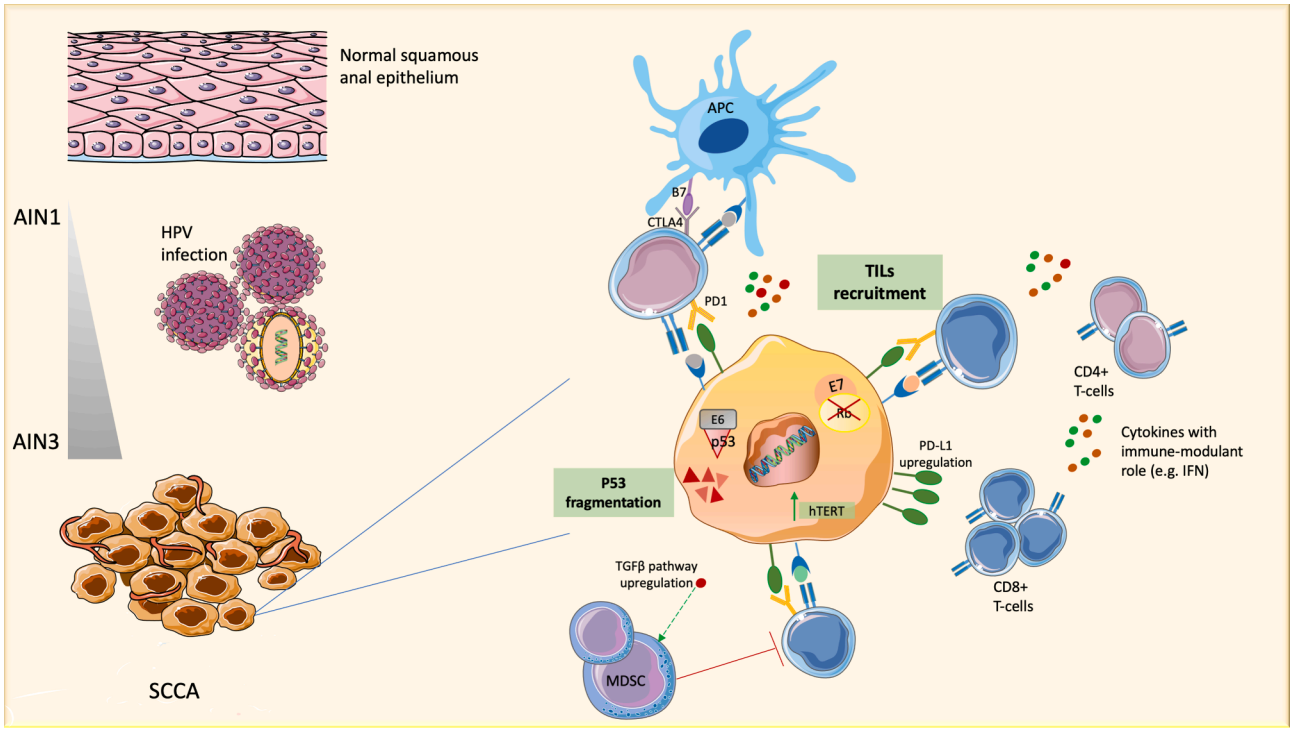

Fig. 1. Effects of human papillomavirus (HPV) on anal cancer immunology. Most squamous cell carcinomas of the anus (SCCA) are caused by HPV infection. HPV integrates into the host DNA or is maintained as extra-chromosomal epitopes, inducing the neoplastic transformation of normal squamous anal epithelium towards the anal intraepithelial neoplasia (AIN 1 to 3) subsequently the SCCA. HPV puts in place several mechanisms of immune evasion: DNA integration to evade host immune response; up-regulation of immune-suppressive pathways, such as Programmed Death-Ligand 1 (PD-L1) or Transforming growth factor beta (TGFβ). TGFβ induces Myeloid-derived suppressor cells (MDSC), promoting Tregs over T effectors. HPV genome promotes the expression of specific proteins, such as E6 and E7. E6 induces p53 fragmentation, E7 blocks Rb, both E6 and E7 promote Telomerase reverse transcriptase (hTERT) reactivation, leading to cancer cell immortalization, neoplastic growth and spread. Moreover, E6 and E7 stimulate specific CD4 + and CD8 + responses recruiting Tumor-infiltrating lymphocytes (TILs) at tumor sites.

regulation [4,5,9,17,19,22,23].

Regardless of its status, the viral genome promotes the expression of E6 and E7 proteins which inhibit p53 and pRb [4,21]. These events correlate with compensatory upregulation of p16 which is a common surrogate at immunostaining of HPV infection: moderate or strong p16 reactivity are associated with better response to CRT and outcomes than a weak or absent expression of p16 [10,24,25].

Balermpas and colleagues demonstrated that patients with higher HPV viral load (and higher p16 immunohistochemical expression) had better outcomes after CRT compared to patients with low viral load and absence of p16 immunohistochemical expression [26]. This finding relies on the association between a higher viral load and a greater intratumoral (not stromal) CD8 + PD-1 + tumor infiltrating lymphocytes (TIL) expression, thus supporting the idea that HPV may render tumors more immunogenic [26].

It is worth noting that HPV16 E6 and E7 proteins induce a specific T-helper 1 Th1 CD4 and T-cytotoxic CD8 restricted response in peripheral blood and in the tumor microenvironment (TME), responsible for TIL recruitment [7,13]. Moreover, E6 promotes the reactivation of hTERT (human telomerase reverse transcriptase) gene transcription with the immortalization of cancer cells. Interestingly, Th1-CD4 cells restricted for hTERT have been detected in SCCA patients and may have a prognostic value [7,13,14].

Epitopes-HPV01 and Epitopes-HPV02 trials demonstrated that only the anti-hTERT Th1-CD4 cell response, evaluated after chemotherapy, was related to clinical outcomes. DCF and mDCF were able to enhance immune responses which may contribute, particularly that restricted for hTERT, to the duration of clinical responses [27].

The role of PD-L1 in SCCA

PD-L1 status has been widely evaluated both in metastatic and localized SCCA, based on the prominent role of this axis in HPV-driven immune-evasion. Iseas and colleagues described PD-L1 combined positive score (CPS) > 1 %in almost 57% of cases of localized SCCA samples with: higher PD-L1 expression levels was associated with increased CD3 and CD8 TILs, higher rates of response, significantly better OS compared to PD-L1 CPS < 1% patients [8]. Similar PD-L1 expression levels were described in metastatic disease and a higher density of CD8 TIL was demonstrated even in the metastatic setting among PD1-responders compared to non-responders [8,17,18]. Several factors may be responsible for modulating PD-L1 levels: IFN-γ secretion, PI3K/AKT/mTOR pathway as well as PTEN activity.

It has been reported that a high expression of PD-L1 in anal cancer could be related to the immune response against HPV E6 and E7 oncoproteins: high levels of IFN-γ secreted by TILs could upregulate PD-L1 leading to the so-called “adaptive immune resistance” [17].

On the other hand, it has been shown that PD-L1 expression and HPV infection were not correlated: no significant differences in HPV infection status were detected in tumors expressing PD-L1 compared to those PD-L1 negative, thus hypothesizing that PDL-1 is an independent prognostic marker in SCCA, associated with better survival [21]. Furthermore, PD-L1 might represent a predictive biomarker for SCCA and provided the rationale for implementing therapeutic strategies targeting the PD-1/PD-L1 axis both in non-metastatic and metastatic SCCA. Of note, recent studies suggest that HIV status does not affect the degree or composition of immune cell infiltrate or PD-L1 expression in SCCA [26,28].

Comprehensive genome profiling and the advent of molecular characterization

The use of comprehensive genome profiling (CGP) techniques on patient’s samples of SCCA detected a high rate of somatic alterations in genes related to PI3K/AKT/mTOR pathway: PIK3CA activating mutations (16%-40%), recurrent amplifications of the locus harboring this gene (57% of cases), homozygous deletions of PTEN (15% of all the cases), alterations in FBXW7, RICTOR and STK11 genes [4,8,20,29,30]. Chung and colleagues demonstrated their presence both in HPV-positive and negative SCCA, irrespective of disease stage: this latter evidence suggests they may represent an early event in SCCA tumorigenesis [29].

Of note, PI3K/AKT/mTOR pathway represents one of the mechanisms able to induce PD-L1 up-regulation in tumor cells. Altogether, these data suggest its pivotal role in SCCA pathogenesis as well as point out a new potential therapeutic target to explore [4,8,29].

While, epidermal growth factor receptor (EGFR) alterations were described only in a small fraction of SCCA, high rates of immunohistochemical expression have been reported in most of them [29,31,32].

CGP studies also showed a significant enrichment in tp53 and CDKN2A alterations in HPV negative-tumors [29]. These findings are biologically plausible given that HPV oncogenesis relies predominantly on p53 and pRb inactivation. Inactivating mutations of CDKN2A gene may explicate the negative immunostaining for p16 reported in a significant proportion of HPV-negative SCCA. Finally, tumors bearing tp53 mutations showed a typical hyperactivation of mTOR signaling [4,8].

Loss of PTEN has been described as a frequent alteration in SCCA [33,34]. Data deriving from cancer models different from anal cancer suggest that its loss exerts a predominantly immunosuppressant activity through several concomitant events: impaired activation of type I-IFN pathway; increased M2-like macrophages, T reg cells and myeloid derived suppressor-cells (MDSCs) density in TME and PD-L1 overexpression [33,34].

The emerging immunomodulatory role of myeloid derived suppressor cells

Finally, the role of myeloid derived suppressor cells (MDSCs) has been explored in anal cancer. MDSCs comprise a heterogeneous population of myeloid progenitors and immature cells which suppress antigen-presenting cells (APC) and T-cell responses [7]. Interestingly, Epitopes HPV01 and HPV02 studies demonstrated that Monocytic-MDSC (M−MDSC) play a major prognostic role in advanced SCCA patients by modulating the intensity and frequency of hTERT immune responses and that DCF could deplete them, thus restoring anti-tumor immune competence [7,13,14,27,35].

Completed studies

Based on the strong biological rationale, several clinical trials investigated the role of immunotherapy in advanced SCCA (Table 1).

ICIs alone

In a phase II single-arm (NCT02314169), 37 patients with SCCA, irrespective of PD-L1 expression, received nivolumab as second line or subsequent treatment [18]. HIV-positive patients with controlled disease were eligible. An ORR of 24% was reported, with 2 complete responses (CR) and 7 partial responses (PR). Median PFS (mPFS) and mOS were 4.1 and 11.5 months respectively. The safety profile was judged acceptable, with 5 grade 3 adverse events (AEs) and no grade 4 AEs [18]. HPV was detected in all the pre-existing tumor samples [15]. Pretreatment tumor samples showed higher baseline percentages of T cells expressing CD8 and granzyme B in responders compared to non-responders, as well as higher expression of PD-1 on TIL and PD-L1 on tumor cells. Furthermore, at flow cytometry, responders had higher PD-1 expression on CD8 T cells and greater co-expression of LAG-3 and TIM-3 compared to non-responders [18,36].

Phase Ib KEYNOTE-028 (NCT02054806) was a multicenter, multicohort, single-arm trial that evaluated pembrolizumab monotherapy in patients with 20 different PD-L1-positive tumor types [17]. In the cohort of patients with advanced heavily pretreated anal cancer, among the 24 patients with squamous histology, 4 obtained a confirmed PR, for an ORR equal to 17%. Median PFS and OS were 3.0 and 9.3 months

Table 1
Completed clinical trials assessing the use of immune checkpoint inhibitors (ICIs) for the treatment of squamous cell carcinoma of the anus (SCCA).

Study Name	Agent	Target	Phase	Patients	Setting	Outcomes
ICIs alone						
NCT 02,314,169 (NCI 9673)	Nivolumab 3 mg/kg q14	PD-1	II	37	second line or subsequent	ORR: 24% mPFS: 4.1 m mOS: 11.5 m
NCT02054806 (Keynote 028) SCCA cohort	Pembrolizumab 10 mg/kg q14	PD-1	Ib	25 (24 with SCCA)	second line or subsequent	ORR: 17% mPFS: 3.0 m mOS:9.3 m
NCT02628067 (Keynote 158) SCCA cohort	Pembrolizumab 200 mg q21	PD-1	II	112	second line or subsequent	ORR:11.6% mPFS: 2.0 m mOS: 12.0 m
NCT03597295 (POD1UM 202)	Retifanlimab	PD-1	II	94	second line or sebsequent	ORR: 13.8% mPFS: 2.3 m mOS: 10.1 m
ICIs + EGFR inhibitors						
NCT03944252 (CARACAS)	Avelumab + cetuximab (arm B) vs avelumab alone (arm A)	PD-L1 EGFR	II	60	second line or subsequent	ORR: 17% vs 10% mPFS: 3.9 vs 2.0 m mOS: 7.8 vs 13.9 m (ARM B vs ARM A)
Vaccines alone or combined with ICIs						
NCT02426892 HPV 16-positive cancers	ISA101 + nivolumab	HPV16 peptide vaccine PD-1	II	24 (1 with anal cancer)	second line or subsequent	ORR of 33% mPFS: 2.7 m mOS: 17.5 m
NCT02399813	ADXS11-001 monotherapy	HPV 16 E7 protein	II stage I	29	second line or subsequent	ORR. 3.4% 6-month-PFS rate: 15.5%.
Adoptive T cell therapy						
NCT01585428 HPV-related cancers	Autologous HPV TILs		II	29 (5 with anal cancer)	second line or subsequent	ORR: 18% (non cervical cohort)
NCT02280811 HPV 16 -positive cancers	E6 TCR T cells	E6	i/II	12 (4 with anal cancer)	second line or subsequent	DLT: none MTD: $105x10^9$ ORR:2/12 pts
NCT02858310 HPV16-positive cancers	E7 TCR T cells	E7	I/II	12 (2 with SCCA)	second line or subsequent	MDT: 100 billion E7 TCR T cells ORR: 6/12 pts
Dual PD-L1 and TGFβ blockade						
NCT02517398 NCT03427411 HPV associated cancers	Bintrasfusp alfa alone	PD-L1 TFG-β	Ib II	59 (6 with SCCA)	second line or subsequent	ORR: 30.5% mPFS: 2.8 m mOS not reached
Other combinatory strategy						
NCT03074513 Anal cancer cohort	Atezolizumab + bevacizumab	PD-L1 VEGF-A	II	20	second line or subsequent	ORR: 10% mPFS: 4,1m mOS: 11,6m
NCT03517488 (DUET 2)	XmAb®20717	PD-1 CTLA-4	I	109	2 nd line or later	MTD 10 mg/kg ORR:13% (21% at 10 mg/kg)

PD-1: programmed death 1; PD-L1: programmed death ligand 1; EGFR: epidermal growth factor receptor; TGF-β: transforming growth factor beta; VEGF-A: vascular endothelial growth factor A;CTLA-4: cytotoxic T-lymphocyte-associated antigen 4; ORR: overall response rate; mPFS: median progression free survival; mOS: median overall survival; DLT: dose limiting toxicity; MTD: maximum dose tolerated.

respectively, similar to those reported with nivolumab, while median duration of response (DOR) was not reached at the time of analysis [17,37].

The non-randomized, multicohort, phase 2 trial KEYNOTE-158 (NCT02628067) evaluated pembrolizumab in patients with previously treated, advanced cancers, irrespective of PD-L1 expression [38]. The final data of the SCCA cohort have been recently published: 112 patients with documented metastatic and/or unresectable SCCA with prior treatment failure received single-agent pembrolizumab. ORR was 11 % (6 CR; 6 PR): patients with PD-L1 CPS $\geq$ 1 (assessed with PD-L1 IHC 22C3 pharmDx assay) had higher ORR (11/75) compared to the PD-L1 CPS $<$ 1 counterpart (1/30) (15% vs 3%), although no significant difference in OS and PFS among the two groups was described. mOS was 11.9 months, mPFS was 2.0 months while median DOR was not reached. [19,38] However, the single arm study design precludes interpretation of whether PDL1 expression might affect mOS and mPFS exerting a prognostic or predictive value. Safety profile was consistent with previous reports. [17,18].

A pooled analysis of the SCCA cohorts of both KEYNOTE-028 and KEYNOTE-158 trials corroborated these data [39]. 137 treated patients (25 in KEYNOTE-028 and 112 in KEYNOTE-158) were included (73.0% had PD-L1–positive tumors). ORR in the whole population was 10.9% (8 CR and 7 PR) median DOR was not reached. Median PFS and mOS were 2.1 months and 11.7 months respectively [38,39].

In the POD1UM 202 (NCT03597925), a phase II, single-arm study, 94 patients with previously pre-treated advanced SCCA, received retifanlimab, an anti-PD-1 antibody, regardless of PD-L1 status [40]. HIV patients with controlled disease could be included. ORR was 13.8% (1CR and 12 PR) with no difference related to HIV-positive or PDL-1 status. MPFS and mOS were 2.3 and 10.1 months respectively, with a median DOR of 9.5 months [40].

Despite the strong rationale and the promising results, anti-PD1 treatment alone induces a limited number of responses, thus encouraging the use of combination strategies.

ICI + anti EGFR

A huge amount of evidence suggests that the anti-EGFR cetuximab could elicit the immune response by inducing natural killer (NK) cells driven antibody-dependent cytotoxicity (ADCC) [41,42]. It has been

recently reported that combining cetuximab with the anti PD-L1 avelumab could determine prolonged survival in pre-treated patients with colorectal cancer and non-small cell lung cancer [42-44]. In the CARACAS study, an open-label, multicenter, randomized, phase II trial (NCT03944252), 60 patients diagnosed with metastatic SCCA progressing after one or more lines of treatment were randomized (1:1 ratio) to receive avelumab alone (arm A) or combined with cetuximab (arm B) [45]. 4 patients were HIV-positive. Primary endpoint was ORR, and at least 4 responses out of 27 patients per arm had to be observed to declare the study positive. The primary endpoint was met in arm B, with an ORR of 10% in arm A and 17% in arm B. As to the secondary endpoints, mPFS was 2.0 months and 3.9 months, while mOS was 13.9 months and 7.8 months in arm A and B respectively. This latter result should be interpreted cautiously due to the non-comparative design of the trial, the the small size of the sample and the potential confounding effects of some unbalanced prognostic factors.

Therapeutic cancer vaccines

Vaccines have been evaluated both alone and in combination with anti-PD1 agents. Being PD1/PD-L1 blockade not enough to achieve durable and complete responses in most patients, anti-cancer vaccines may enhance its efficacy by activating tumor-specific T cells [46]. In the meantime, ICIs may augment vaccine-induced immune responses by modulating the immunosuppressive tumor environment [19]. In the single-arm, single-center, phase II NCT02426892 trial, 24 patients with recurrent/metastatic HPV16-positive cancers (22 oropharyngeal, 1 anal and 1 cervical) received ISA101, a synthetic peptide HPV16 vaccine able to induce HPV-specific T cells, in combination with nivolumab [46]. The ORR was 33% (8/24 patients, all with oropharyngeal carcinoma). m PFS and mOS were 2.7 and 17.5 months respectively. However, no meaningful efficacy conclusions can be made on metastatic SCCA patients as just one patient with this diagnosis was enrolled and had progressive disease [46].

NCT02399813 was a Simon 2-stage, single-arm, multicenter, phase 2 trial, that evaluated ADXS11-001 monotherapy, in patients with persistent/recurrent or metastatic previously treated SCCA. ADXs11-00 vaccine is an attenuated Listeria Monocytogenes strain bioengineered to secrete a fusion protein containing the HPV-16 E7 oncoprotein: phagocytosis results in presentation to E7-restricted T cells with consequent activation and antibody production [47]. 29 patients out of 36 treated were evaluable for response: stage I ORR was 3.4% (only one prolonged PR after failure of ICI) while 6-month-PFS rate was 15.5%. Although ADXS11-001 was safe, the co-primary endpoints for stage I in the Simon 2-stage design (ORR $\geq$ 10% or 6-month PFS rate $\geq$ 20%) were not met to proceed to the second phase [47].

Adoptive T cell therapy

Adoptive T cell Therapy (ACT), the systemic infusion of therapeutic T cells, is an emerging cancer treatment option in patients with gastrointestinal malignancies [48].

NCT01585428 is a phase II trial that tested the infusion of autologous TIL, derived from tumor fragments, preceded by lymphodepletion and followed by systemic injection of high-dose aldesleukin in patients with HPV-related metastatic/recurrent cancers [49]. Overall, 29 patients were recruited in two cohorts: cervical cancers (18 patients) and non-cervical cancers (11 patients, 5 with anal cancer). The experimental treatment showed modest clinical activity: ORR was 28% in the cervical cancer cohort and 18% (2/11 patients) in the non-cervical cancer cohort. Interestingly, one patient with progressing anal cancer with lung metastases attained a PR that lasted 4 months. HPV-TILs displayed greater frequencies of HPV-reactive T cells and higher concentrations of HPV-specific IFN-γ release in responders versus non-responding patients [49].

Recent strategies that generate a more reactive HPV oncoprotein-targeted cell population, include the administration of peripheral blood HPV-specific T cells that are propagated ex vivo, or the use of peripheral blood T cells that are genetically engineered ex vivo to target HPV oncoproteins.

In the NCT02280811, phase I/II, single-center trial, 12 patients with metastatic HPV16-positive cancers from several primary tumor sites (4 anal), who had been treated with prior platinum-based therapy, received an infusion of autologous genetically engineered T cells expressing high-avidity T-cell receptor directed against an HLA-A*0201-restricted HPV16 E6 epitope (E6 T-cell receptor T cells) [50]. Two patients out of 12 with SCCA experienced a PR: a 48-year-old woman with lung progression who had already received chemotherapy and HPV-TILs, and a 64-year-old woman previously treated with chemotherapy and nivolumab. Despite targeting a tumor antigen that should be constitutively expressed with a high-avidity T-cell receptor (TCR), response rate was modest: several mechanisms of resistance were identified such as mutations in IFNGR1, loss of HLA-A*02:01, expression of PD-1 by tumor-infiltrating E6 TCR-T cells, and expression of PD-L1 by tumor-infiltrating immune cells [50].

The first results of the phase I/II, open label NCT02858310 trial were recently published [51]. 12 HLA-A*02:01-positive patients with metastatic HPV-16 + cancers, previously treated with standard regimens, received an infusion of autologous genetically changed T cells restricted for HPV16-E7 oncoprotein (E7 TCR cells). Of note, 2 patients had metastatic SCCA. The primary endpoint was the maximum tolerated dose (100 billion E7 TCR-T cells). Six out of 12 patients attained PR, including 4 of 8 patients with anti-PD-1 refractory disease: 1 with metastatic SCCA and lesions in the thorax, retroperitoneum, bones, and kidney, previously treated with chemoradiation and anti-PD-1 therapy, experienced a PR lasting 9 months. The ORR reported was higher than that observed in NCT02280811 trial with E6 TCR T cells in a similar setting: this may suggest higher functional avidity and greater anti-tumor functions of E7 TCR T cells than E6 TCR T cells [51].

Dual PD-L1 and TGFβ blockade

An association between HPV infection and the upregulation of TGFβ pathway has been described [23]. In preclinical studies, dual TGFβ and PD1/PD-L1 blockade enhanced antitumor activity with decreased regulatory T cell function, reduced MDSC infiltration, and increased NK cells and TCD8 cells densities [23,52,53]. Based on this rationale, different studies evaluated the combination of TGFβ inhibitors and ICIs in different tumor types including anal cancer [54]. The phase 1 NCT02517398 and phase 2 NCT03427411 trials investigated the safety and efficacy of bintrafusp alfa (M7824), a bifunctional fusion protein consisting of an anti PD-L1 antibody bound to the extracellular domain of the human TGFβ receptor 2, in several HPV-associated cancers [23]. A first post-hoc analysis included 59 patients diagnosed with advanced, pretreated, ICI-naïve, HPV-associated cancers: 6 patients had metastatic SCCA. The confirmed ORR in the checkpoint inhibitor-naïve full-analysis population was 30.5%: 2 patients with SCCA attained CR and PR respectively. As to safety, 27,1% of patients experienced grade 3–4 adverse events. In the full-analysis set, mPFS was 2.8 months while mOS was not reached. The full analysis set did not include the 20 patients refractory to ICIs enrolled in the phase 2 study. Moreover, the small number of patients with SCCA limits the possibility to make significant conclusions [23].

At ESMO Congress 2021, the authors reported longer follow-up of additional patients pooled from these studies. A total of 75 patients received bintrafusp alfa in the phase I and phase II studies: among them, 9 patients had anal cancer. The ORR was 28.0% (4 CR and 17 PR, with 2 PR in SCCA). Median DOR was 17.3 months while mOS was 21.3 months [55].

Other combinatory strategies

Given the role played by angiogenesis in immune evasion mechanisms, the phase II basket-trial NCT03074513 evaluated the combination of the anti PD-L1 atezolizumab plus bevacizumab in patients with previously treated, immunotherapy-naïve, HPV-associated solid tumors [56]. Results for SCCA patients were presented at ESMO 2021: 20 patients with unresectable SCCA were enrolled. ORR was 10% (2 PR). mPFS and mOS were 4.1 months and 11.6 months respectively.

Finally, DUET 2 (NCT03517488) is an ongoing, multiple-dose, phase 1 trial investigating XmAb®20717, a humanized bispecific monoclonal antibody that simultaneously targets PD-1 and CTLA-4, in patients with advanced solid tumors, including SCCA, progressed to standard therapies [57]. Preliminary results on 109 patients were recently published. 10 mg/kg was identified as the recommended dose. ORR was 13% (1 CR and 5 PR) in the whole study, and 21% at the recommended dose of 10 mg/kg. Of note, responses were observed only within the 10 mg/kg group. A good safety profile was reported. Unfortunately, in the preliminary analysis, no information about SCCA patients were available [57].

Possible biomarkers to optimize patient selection and treatment efficacy

Anti PD-1/PD-L1 single agent displayed a limited anti-tumor activity in un-selected patients with SCCA. Therefore, patients' selection for immune checkpoint blockade still represents an unmet need [45]. Selection is closely related to the urgency of more translational research to detect biomarkers that may predict who really benefit from PD-1/ PD-L1 blockade and identify driver mutations that may underlie immunogenicity.

The role of HPV infection and viral load has been considered, being most SCCA ($\geq$80%) HPV-related and being HPV closely associated with immunogenicity. However, HPV infection predictive role in immune checkpoint blockade has not been fully explored in SCCA [23,45].

Interestingly, a common finding in responders was represented by higher densities of CD3 and CD8 TIL [7,8]. In the NCI9673 trial, a higher percentage of TCD8 infiltrating cells was detected in pretreatment tumor samples of responding patients compared to non-responders [18].

The role of PD-L1 expression as a biomarker of response in SCCA is a matter of debate. In this regard, PD-L1 expression was not an inclusion criterion in all the trials reported. However, even if not assessed for enrollment, it was evaluated as an exploratory biomarker. In SCCA, ICIs antitumor activity was demonstrated irrespective of PD-L1 status.

In the NCI9673 trial, higher expression of PD-1 on TIL and PD-L1 on tumor cells was reported in responders compared to non-responders [18]. Similarly, a trend towards a higher ORR in patients with PD-L1 CPS $>$ 1 compared to those with CPS $<$ 1 was confirmed by a pooled analysis of the SCCA cohorts of both KEYNOTE-028 and KEYNOTE-158 [38,39].

Being HPV products immunogenic and able to evoke specific adaptive immune responses, T cell responses restricted to HPV E6/E7 oncoproteins and hTERT have also been considered as possible biomarkers since their first evaluation in the Epitope HPV-01 and HPV-02 trials [7,12-14,27]. Interestingly, chemotherapy showed to enhance immunity and a better prognosis was associated with hTERT CD4 Th1 response and not with anti HPV E6/E7 immunity [7].

Besides the role of PD-1/PD-L1 axis, other immune checkpoints have been investigated as therapeutic target [5,36].

In the NCI9673 trial, a higher pretreatment co-expression of inhibitory immune markers such as TIM-3 and LAG-3 on PD-1 positive CD8 cells was described in responding patients and associated with durable response to nivolumab [18]. Interestingly, also in the NCT02858310 trial, a higher expression of LAG-3 and TIM-3 receptors was detected on E7 TCR-T cells and decreased with following cell infusions [51]. However, this finding did not correlate with treatment response.

Finally, M−MDSC may represent another intriguing biomarker to predict clinical outcomes and immunotherapy efficacy in SCCA patients. Several studies suggest a possible prognostic value, that must be confirmed in prospective trials [7,14,27,47].

Discussion and future perspectives

Research advances on SCCA have been slow due to several limitations. One of the main obstacles is the low number of patients due to the rarity of the disease that does not facilitate bigger trials exclusively dedicated to SCCA. Most of the data on immunotherapy discussed above derive from multicohort studies designed for HPV-related malignancies in general, in which the number of SCCA patients enrolled is often too limited to extrapolate conclusions. Moreover, only the NCI9673, POD1UM 202, CARACAS and NCT02399813 studies recruited exclusively SCCA patients [18,40,45,47].

Several ongoing trials are still evaluating immune checkpoint inhibitors as second-line strategies or further as well as their earlier use in metastatic SCCA management predominantly in combination with other agents (Table 2).

Regarding localized disease, anti-PD1/L1 agents are potential candidates even in this setting as long-lasting complete responses were seen in chemorefractory patients in advanced SCCA. As already mentioned above, being SCCA an immunogenically "hot" tumor, the combination of CRT with immunotherapeutic approaches may result in improved tumor control and longer clinical responses compared to CRT alone.

Being DCF an effective backbone chemotherapy to combine with anti-PD1/L1 drugs, the phase II INTERACT-ION is now evaluating the efficacy and safety of DCF plus ezabenlimab, an anti-PD1 antibody, and intensity modulated RT (IMRT) as neoadjuvant strategy in stage III SCCA patients (NCT04719988).

Similarly, the CORINTH trial, a phase Ib/II study, is exploring the safety and efficacy of pembrolizumab in combination with concurrent chemotherapy (mitomycin plus 5FU or capecitabine) and IMRT in HPV positive stage IIIA and IIIB SCCA patients (NCT04046133).

Moreover, the BrUOG276 study (NCT01671488) is a phase I/II trial that is exploring the combination of radiotherapy with standard chemotherapy (MMC + 5FU) and ADXS11-01.

Other ongoing trials are evaluating the efficacy and toxicity of CRT with immunotherapy either in the adjuvant setting alone, such as the NCT03233711 phase III trial with nivolumab in patients diagnosed with stage II-III B anal cancer, or in both, the concurrent and the adjuvant setting, such as the RADIANCE trial [58]. With regard to the latter, this prospective, multicenter, randomized phase II study is testing the addition of durvalumab to standard CRT in patients with locally advanced SCCA. In the experimental arm, immunotherapy will start 14 days before initiation of standard CRT and then will be administered every four weeks for a total of 12 doses.

Altogether these studies support the concept that immunotherapeutic approaches play a pivotal role in the overall management of SCCA and not only in the advanced setting, based on the typical immunobiological features of this disease.

As to recurrent/metastatic disease the combination of PD-1/PD-L1 agents with other ICIs is gaining ground as a key strategy to improve the stimulation of the immune response, compared with monotherapies [59,60].

In the phase 2 NCT02314169 trial,137 patients with pretreated metastatic SCCA will receive nivolumab or nivolumab plus the anti-cytotoxic T-Lymphocyte Antigen 4 (CTLA4) ipilimumab. The co-primary endpoints are PFS and ORR [4,5,7,18,36].

Based on the exploratory analyses of the NCI9673 trial showing higher co-expression of TIM-3 and LAG-3 in PD-1 positive tumors responding to nivolumab, the multiple cohort Checkmate 358 (NCT02488759) is evaluating nivolumab alone and nivolumab in combination with ipilimumab, or relatlimab (BMS-986016), an anti-LAG3 agent, or daratumumab, an anti CD38 antibody, in patients with virus-

Table 2
On-going clinical trials.

Study Name	Agent	Target	Phase	Setting	Number	Primary Outcomes
ICIs alone						
NCT02919969	pembrolizumab	PD-1	II	second line or subsequent	32	ORR
NCT02314169 (NCI9673)	nivolumab vs nivolumab + ipilimumab	PD-1 CTLA-4	II	second line or subsequent	137	ORR (part A) PFS (part B)
ICIs plus CT						
NCT04472429 (POD1UM 303)	Carboplatin D1 + paclitaxel D1,8,15 + retifanlimab D1 q28 vs Carboplatin D1 + paclitaxel D1,8,15 + placebo D1 q28	PD-1	III	first line	300	PFS
NCT04444921 (NCI-EA2176)	Carboplatin D1 + paclitaxel D1,8,15 + nivolumab D1,15 q28 (then only in D1) vs Carboplatin D1 + paclitaxel D1,8,15	PD-1	III	first line	205	PFS
NCT03519295 (SCARCE)	atezolizumab + mDCF vs mDCF alone	PD-L1	II	first line	99	12 months- PFS rate
NCT04894370 (SPARTANA)	Spartalizumab + mDCF + SBRT	PD-1	II A	first line	47	12 months-PFS rate
ICIs + Vaccines						
NCT03439085 HPV-associated cancers	INO-3112 + Durvalumab	E6/7 proteins PD-1	II	second line or subsequent	77	ORR
NCT03946358 (volaTIL) HPV positive squamous cell carcinomas	UCPVax + atezolizumab	hTERT PD-L1	II	second line or subsequent	47	ORR at 4 months
NCT04432597 HPV -related cancers	PRGN-2009 + M7824 Vs PRGN-2009 alone	PD-L1 TGF-β	I/II	second line or subsequent	76	1.safety and RP2D of PRGN-2009 2. level increase in CD3 + tumor infiltrating T cells post-treatment compared to pre-treatment
Adoptive T cell therapy alone or plus ICIs						
NCT02379520 (HESTIA Trial) HPV-associated cancers	HPV-16/18 E6/E7-specific autologous T lymphocytes (HPVST cells) alone vs HPVST cells + nivolumab	E6/7 proteins PD-1	I	Second line or later	32	DLT
NCT02858310 HPV 16 + camcer	E7 TCR cells	E7 protein	II	second line or later	180	ORR safety
Others						
NCT02488759 (CHECKMATE 358) Virus-associated tumours	nivolumab alone vs nivolumab + ipilimumab vs nivolumab + relatlimab vs nivolumab + daratumumab	PD-1 CTLA-4 LAG-3 CD-38	I/II	second line or later	584	ORR safety
NCT04499352 Only SCCA	Ezabenlimab alone vs Ezabenlimab + BI 836,880	PD-1 VEGF/ Ang2	II	2 nd line or later	0 (withdrawn for sponsor decision)	ORR

ICIs: immune checkpoint inhibitors; PD-1: programmed death 1; PD-L1: programmed death ligand 1; CTLA-4: cytotoxic T-lymphocyte-associated antigen; hTERT: human telomerase reverse transcriptase; TGF-β: transforming growth factor beta; LAG-3: lymphocyte activating gene 3; CD-38: cluster of differentiation 38; VEGF: vascular endothelial growth factor; Ang2: Angiopoietin- 2; ORR: overall response rate; PFS: progression free survival; RP2D: recommended phase II dose, DLT: dose limiting toxicity

positive and negative tumors including SCCA.

It is well known that chemotherapy can enhance anti-tumor immunity by inducing immunogenic cell death (ICD) and by disrupting tumor immune-escape strategies such elimination of immunosuppressive cells and activation of APC [61-63].

Of note, taxane-based chemotherapy has demonstrated to be effective in SCCA, given that the loss of p53 function, which is a frequent finding in HPV-related SCCA, may confer sensitization to these drugs by increasing G2/M arrest and apoptosis [12,13,64].

In treatment-naive anal cancer patients, two randomized phase III trials are currently evaluating the combination of chemotherapy plus ICIs (Table 2). In the POD1UM-303/InterAACT 2 (NCT04472429) trial, 300 patients with advanced/metastatic SCCA will be randomized to carboplatin and paclitaxel plus retifanlimab or placebo. Similarly, the phase III NCT04444921 study is evaluating the addition of nivolumab to carboplatin and paclitaxel versus chemotherapy alone.

Given the ability of mDCF to promote antitumor immune response through several mechanisms, the SCARCE trial (NCT03519295) is testing the combination of atezolizumab with mDCF compared to mDCF alone [65].

Strong evidence suggests that RT could stimulate the immune response, representing a potential candidate to combine with ICIs [66,67]. The rationale behind the combined use of radiations relies on their ability to modulate the release of tumor antigens, induce MHC class I expression and increase TMB [7,68]. Altogether these phenomena, along with induced PD-L1 expression, could improve anti-PD-1 and anti-PD-L1 efficacy in the irradiated field and even at distance (the so called “abscopal” effect) [69]. In this regard, the phase II SPARTANA trial (NCT04894370) will assess the antitumor activity of combining RT (8 Gy on target lesion), the anti PD-1 spartalizumab, and mDCF in 47 chemo-naïve patients with metastatic SCCA.

Considering the preliminary results and signals of clinical activity, different trials are investigating the association of ICIs with tumor vaccines or adoptive T cell therapy (Table 2) [70].

INO-3112 is a plasmid DNA vaccine that encodes E6 and E7 proteins of HPV16/18 along with IL-12, thus evoking an HPV 16/18 E6- E7 specific immune response. INO-3112 in combination with the anti PD-L1 durvalumab is under evaluation in patients with recurrent/metastatic HPV-associated cancers (including anal cancer) refractory to standard therapies (NCT03439085).

In the NCT04432597 phase I/II trial, another HPV vaccine, PRGN-2009, is under evaluation with or without bintrafusp alfa, in HPV-related cancers, including anal cancer.

Interestingly, as mentioned above, in Epitopes HPV-01 and HPV-02 studies, a significantly better prognosis was related to hTERT immunity and not with HPV E6/7 immune responses, thus suggesting that hTERT vaccine may represent a promising therapeutic option in SCCA patients [7,12-14]. Currently, VolaTIL (NCT03946358) phase II trial is exploring the combination of UCPVax, a vaccine composed of two separate MHC class II-restricted peptides (UCP2 and UCP4) derived from hTERT, and atezolizumab, in locally advanced/metastatic HPV-positive squamous cell carcinomas, including SCCA. Adoptive T cells represent another promising strategy. In the HESTIA phase I trial (NCT02379520), polyclonal HPV-16/18 E6/E7-specific autologous T lymphocytes (HPVST cells) are given intravenously with or without nivolumab in patients with metastatic/relapsed HPV-positive squamous cancers (including SCCA). HPVST cells will be engineered in order to become resistant to TGFβ.

Engineered T cells, particularly E7 TCR T cells, showed robust and persistent activity in HPV + solid tumors even in PD-1 refractory disease. This finding may be explained by the different mechanisms underlying these approaches: E7 TCR-T cells directly target tumors with high-avidity T cells while immune checkpoint blockade indirectly targets tumors through disinhibition of natural T cells, characterized by variable numbers, avidity and specificity [51]. The phase II part of NCT02858310 trial is currently ongoing, to further assess the safety and efficacy of the maximum tolerated dose of E7 TCR cells for the treatment of HLA-A*02:01-positive patients diagnosed with metastatic or recurrent/refractory HPV-16 + cancers [51].

Conclusions

After decades of disappointing results, the therapeutic armamentarium of metastatic anal cancer is rapidly evolving. In this context, PD-1/PD-L1 immune checkpoint is a relevant candidate target for immunotherapy in HPV + cancers, including SCCA. Despite the strong rationale, PD-1/PD-L1 blockade induces only a limited number of long-term responses in SCCA. Therefore, combining anti-PD-1/PD-L1 drugs with other treatments, including chemotherapy, vaccines, adoptive T cells or other ICIs seems to represent a promising strategy. Further translational studies are required to identify the best candidate to benefit from ICIs.

Finally, the results of the ongoing studies are waited and probably will contribute to change clinical practice.

CRediT authorship contribution statement

Davide Ciardiello: Conceptualization, Writing – original draft. **Luigi Pio Guerrera:** Conceptualization, Writing – original draft. **Brigida Anna Maiorano:** Conceptualization, Writing – original draft. **Paola Parente:** Writing – review & editing. **Tiziana Pia Latiano:** Writing – review & editing. **Massimo Di Maio:** Writing – review & editing. **Fortunato Ciardiello:** Writing – review & editing. **Teresa Troiani:** Writing – review & editing. **Erika Martinelli:** Writing – review & editing. **Evaristo Maiello:** Supervision, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Gruppo Oncologico dell'Italia Meridionale (GOIM). We thank Servier Medical Arts for imaging support.

Conflicts of interest

DC has received travel support from Sanofi. TPL has served as speaker for Servier.MdM: Honoraria: Bristol-Myers Squibb, Merck Sharp & Dohme, Pfizer, Takeda Pharmaceuticals, AstraZeneca, Janssen Pharmaceuticals, Mediolanum Farmaceutici, Eisai

Consulting or Advisory Role

AstraZeneca, Merck Sharp & Dohme, Pfizer, Takeda Pharmaceuticals, Janssen Pharmaceuticals, Mediolanum Farmaceutici, Eisai,

Research Funding

Tesaro (Inst). FC has served as advisor and speaker for Roche, Amgen, Merck-Serono, Pfizer, Sanofi, Bayer, Servier, BMS, Cellgene, Lilly. Received institutional Research Grants form Bayer, Roche, Merck-Serono, Amgen, AstraZeneca, Takeda. TT has served as a advisor and speaker for Roche, Merck-Serono, Sanofi, Servier, Novartis, Bayer. ErM has served as advisor and speaker for Astra Zeneca, Amgen, Bayer, Merck-Serono, Roche, Sanofi, Servier, Pierre Fabre. EvM has served as advisor and speaker for Astra Zeneca, Eli Lilly, Servier, Sanofi Genzyme, Roche, Merck, Eisai, Pfizer. All the other authors declare no competing interests.

References

[1] Rao S, Guren MG, Khan K, Brown G, Renehan AG, Steigen SE, et al. Anal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up☆. Ann Oncol. 2021;32(9):1087–100. https://doi.org/10.1016/j.annonc.2021.06.015.
[2] Martin D, Balermpas P, Winkelmann R, Rödel F, Rödel C, Fokas E. Anal squamous cell carcinoma - State of the art management and future perspectives. Cancer Treat Rev. 2018;65:11–21. https://doi.org/10.1016/j.ctrv.2018.02.001.
[3] Pessia B, Romano L, Giuliani A, Lazzarin G, Carlei F, Schietroma M. Squamous cell anal cancer: Management and therapeutic options. Ann Med Surg (Lond) 2020;55:36–46. https://doi.org/10.1016/j.amsu.2020.04.016. Published 2020 May 12.
[4] Carr RM, Jin Z, Hubbard J. Research on Anal Squamous Cell Carcinoma: Systemic Therapy Strategies for Anal Cancer. Cancers (Basel) 2021;13(9):2180. https://doi.org/10.3390/cancers13092180.
[5] Phuong L, Rajdev L. Immunotherapy in Anal Cancer. Curr Oncol Rep 2020;22(9). https://doi.org/10.1007/s11912-020-00946-3.
[6] Martini G, Arrichiello G, Borrelli C, Poliero L, Martinelli E. How I treat anal squamous cell carcinoma. ESMO Open. 2020;4(Suppl 2):e000711. https://doi.org/10.1136/esmoopen-2020-000711.
[7] Spehner L, Boustani J, Cabel L, Doyen J, Vienot A, Borg C, et al. Present and Future Research on Anal Squamous Cell Carcinoma. Cancers 2021;13(15):3895. https://doi.org/10.3390/cancers13153895.
[8] Iseas S, Golubicki M, Robbio J, Ruiz G, Guerra F, Mariani J, et al. A clinical and molecular portrait of non-metastatic anal squamous cell carcinoma. Transl Oncol. 2021;14(6):101084. https://doi.org/10.1016/j.tranon.2021.101084.
[9] Morel A, Neuzillet C, Wack M, Lameiras S, Vacher S, Deloger M, et al. Mechanistic Signatures of Human Papillomavirus Insertions in Anal Squamous Cell Carcinomas. Cancers 2019;11(12):1846. https://doi.org/10.3390/cancers11121846.
[10] Serup-Hansen E, Linnemann D, Skovrider-Ruminski W, Høgdall E, Geertsen PF, Havsteen H. Human papillomavirus genotyping and p16 expression as prognostic factors for patients with American Joint Committee on Cancer stages I to III carcinoma of the anal canal. J Clin Oncol. 2014;32(17):1812–7. https://doi.org/10.1200/JCO.2013.52.3464.
[11] Kim S, Jary M, Mansi L, Benzidane B, Cazorla A, Demarchi M, et al. DCF (docetaxel, cisplatin and 5-fluorouracil) chemotherapy is a promising treatment for recurrent advanced squamous cell anal carcinoma. Ann Oncol. 2013;24(12):3045–50. https://doi.org/10.1093/annonc/mdt396.
[12] Kim S, Jary M, André T, Vendrely V, Buecher B, François E, et al. Docetaxel, Cisplatin, and 5-fluorouracil (DCF) chemotherapy in the treatment of metastatic or unresectable locally recurrent anal squamous cell carcinoma: a phase II study of French interdisciplinary GERCOR and FFCD groups (Epitopes-HPV02 study). BMC Cancer 2017;17(1). https://doi.org/10.1186/s12885-017-3566-0.
[13] Kim S, François E, André T, et al. Docetaxel, cisplatin, and fluorouracil chemotherapy for metastatic or unresectable locally recurrent anal squamous cell

carcinoma (Epitopes-HPV02): a multicenter, single-arm, phase 2 study. Lancet Oncol. 2018;19(8):1094–106. https://doi.org/10.1016/S1470-2045(18)30321-8.
[14] Kim S, Meurisse A, Spehner L, Stouvenot M, François E, Buecher B, et al. Pooled analysis of 115 patients from updated data of Epitopes-HPV01 and Epitopes-HPV02 studies in first-line advanced anal squamous cell carcinoma. 175883592097535 Ther Adv Med Oncol 2020;12. https://doi.org/10.1177/1758835920975356.
[15] Rao S, Sclafani F, Eng C, Adams RA, Guren MG, Sebag-Montefiore D, et al. International Rare Cancers Initiative Multicenter Randomized Phase II Trial of Cisplatin and Fluorouracil Versus Carboplatin and Paclitaxel in Advanced Anal Cancer: InterAAct. J Clin Oncol. 2020;38(22):2510–8. https://doi.org/10.1200/JCO.19.03266.
[16] Stouvenot M, Meurisse A, Saint A, Buecher B, André T, Samalin E, et al. Second-line treatment after docetaxel, cisplatin and 5-fluorouracil in metastatic squamous cell carcinomas of the anus. Pooled analysis of prospective Epitopes-HPV01 and Epitopes-HPV02 studies. Eur J Cancer 2022;162:138–47. https://doi.org/10.1016/j.ejca.2021.11.019.
[17] Ott PA, Piha-Paul SA, Munster P, Pishvaian MJ, van Brummelen EMJ, Cohen RB, 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):1036–41. https://doi.org/10.1093/annonc/mdx029.
[18] Morris VK, Salem ME, Nimeiri H, Iqbal S, Singh P, Ciombor K, et al. Nivolumab for previously treated unresectable metastatic anal cancer (NCI9673): a multicentre, single-arm, phase 2 study. Lancet Oncol. 2017;18(4):446–53. https://doi.org/10.1016/S1470-2045(17)30104-3.
[19] Farias JPF, Rangel da Silva MHC, Jácome AA. Emerging and Experimental Agents for Anal Cancer: What is New? J Exp Pharmacol. 2021;13:433–40. https://doi.org/10.2147/JEP.S262342. Published 2021 Apr 9.
[20] Morris V, Rao X, Pickering C, Foo WC, Rashid A, Eterovic K, et al. Comprehensive Genomic Profiling of Metastatic Squamous Cell Carcinoma of the Anal Canal. Mol Cancer Res. 2017;15(11):1542–50. https://doi.org/10.1158/1541-7786.MCR-17-0060.
[21] Wessely A, Heppt MV, Kammerbauer C, Steeb T, Kirchner T, Flaig MJ, et al. Evaluation of PD-L1 Expression and HPV Genotyping in Anal Squamous Cell Carcinoma. Cancers 2020;12(9):2516. https://doi.org/10.3390/cancers12092516.
[22] Sharma S, Leung WK, Heslop HE. Virus-specific T cells for malignancies - then, now and where to? Curr Stem Cell Rep 2020;6(2):17–29. https://doi.org/10.1007/s40778-020-00170-6.
[23] Strauss J, Gatti-Mays ME, Cho BC, Hill A, Salas S, McClay E, et al. Bintrafusp alfa, a bifunctional fusion protein targeting TGF-β and PD-L1, in patients with human papillomavirus-associated malignancies. J Immunother Cancer 2020;8(2):e001395. https://doi.org/10.1136/jitc-2020-001395.
[24] Meulendijks D, Tomasoa NB, Dewit L, Smits PHM, Bakker R, van Velthuysen M-L F, et al. HPV-negative squamous cell carcinoma of the anal canal is unresponsive to standard treatment and frequently carries disruptive mutations in TP53. Br J Cancer 2015;112(8):1358–66. https://doi.org/10.1038/bjc.2015.20.
[25] Jones CM, Goh V, Sebag-Montefiore D, Gilbert DC. Biomarkers in anal cancer: from biological understanding to stratified treatment. Br J Cancer 2017;116(2):156–62. https://doi.org/10.1038/bjc.2016.398.
[26] Balermpas P, Martin D, Wieland U, Rave-Fränk M, Strebhardt K, Rödel C, et al. Human papilloma virus load and PD-1/PD-L1, CD8+ and FOXP3 in anal cancer patients treated with chemoradiotherapy: Rationale for immunotherapy. Oncoimmunology 2017;6(3):e1288331. https://doi.org/10.1080/2162402X.2017.1288331.
[27] Spehner L, Kim S, Vienot A, François E, Buecher B, Adotevi O, et al. Anti-Telomerase CD4+ Th1 Immunity and Monocytic-Myeloid-Derived-Suppressor Cells Are Associated with Long-Term Efficacy Achieved by Docetaxel, Cisplatin, and 5-Fluorouracil (DCF) in Advanced Anal Squamous Cell Carcinoma: Translational Study of Epitopes-HPV01 and 02 Trials. IJMS 2020;21(18):6838. https://doi.org/10.3390/ijms21186838.
[28] Yanik EL, Kaunitz GJ, Cottrell TR, Succaria F, McMiller TL, Ascierto ML, et al. Association of HIV Status With Local Immune Response to Anal Squamous Cell Carcinoma: Implications for Immunotherapy. JAMA Oncol. 2017;3(7):974. https://doi.org/10.1001/jamaoncol.2017.0115.
[29] Chung JH, Sanford E, Johnson A, Klempner SJ, Schrock AB, Palma NA, et al. Comprehensive genomic profiling of anal squamous cell carcinoma reveals distinct genomically defined classes. Ann Oncol. 2016;27(7):1336–41. https://doi.org/10.1093/annonc/mdw152.
[30] Mouw KW, Cleary JM, Reardon B, Pike J, Braunstein LZ, Kim J, et al. Genomic Evolution after Chemoradiotherapy in Anal Squamous Cell Carcinoma. Clin Cancer Res. 2017;23(12):3214–22. https://doi.org/10.1158/1078-0432.CCR-16-2017.
[31] Lê LH, Chetty R, Moore MJ. Epidermal growth factor receptor expression in anal canal carcinoma. Am J Clin Pathol 2005;124(1):20–3. https://doi.org/10.1309/X4UADHVN317V2XMW.
[32] Rogers JE, Ohinata A, Silva NN, Mehdizadeh A, Eng C. Epidermal growth factor receptor inhibition in metastatic anal cancer. Anticancer Drugs 2016;27(8):804–8. https://doi.org/10.1097/CAD.0000000000000383.
[33] Wu Y, Chen W, Xu ZP, Gu W. PD-L1 Distribution and Perspective for Cancer Immunotherapy—Blockade, Knockdown, or Inhibition. Front. Immunol 2019;10. https://doi.org/10.3389/fimmu.2019.02022.
[34] Vidotto T, Melo CM, Castelli E, Koti M, Dos Reis RB, Squire JA. Emerging role of PTEN loss in evasion of the immune response to tumours. Br J Cancer 2020;122(12):1732–43. https://doi.org/10.1038/s41416-020-0834-6.
[35] Borg C, Spehner L, Kroemer M, Kim S, Jary M, Vienot A, et al. Myeloid derived suppressor cells but not regulatory T cells are associated with adaptive immunity and clinical outcomes in anal squamous cell carcinoma. Ann Oncol 2019;30:v770–1.
[36] Morris VK, Eng C. Role of Immunotherapy in the Treatment of Squamous Cell Carcinoma of the Anal Canal. J Natl Compr Canc Netw 2018;16(7):903–8. https://doi.org/10.6004/jnccn.2018.7040.
[37] Bian JJ, Almhanna K. Anal cancer and immunotherapy—are we there yet? Transl Gastroenterol Hepatol 2019;4:57. https://doi.org/10.21037/tgh10.21037/tgh.2019.08.02.
[38] Marabelle A, Cassier PA, Fakih M, Kao S, Nielsen D, Italiano A, et al. Pembrolizumab for previously treated advanced anal squamous cell carcinoma: results from the non-randomised, multicohort, multicentre, phase 2 KEYNOTE-158 study. Lancet Gastroenterol Hepatol 2022. https://doi.org/10.1016/S2468-1253(21)00382-4.
[39] Marabelle A, Cassier PA, Fakih M, Kao S-C-H, Nielsen D, Italiano A, et al. Pembrolizumab for previously treated advanced anal squamous cell carcinoma: Pooled results from the KEYNOTE-028 and KEYNOTE-158 studies. J Clin Oncol 2020;38:4020.
[40] Rao S, Capdevila J, Gilbert D, Kim S, Dahan L, Kayyal T, et al. LBA42 POD1UM-202: phase II study of retifanlimab in patients (pts) with squamous carcinoma of the anal canal (SCAC) who progressed following platinum-based chemotherapy. Ann Oncol 2020;31:S1170–1. https://doi.org/10.1016/j.annonc.2020.08.2272.
[41] Bourhis J, Stein A, Paul de Boer J, Van Den Eynde M, Gold KA, Stintzing S, et al. Avelumab and cetuximab as a therapeutic combination: An overview of scientific rationale and current clinical trials in cancer. Cancer Treat Rev 2021;97:102172. https://doi.org/10.1016/j.ctrv.2021.102172.
[42] Fasano M, Della Corte CM, Di Liello R, Barra G, Sparano F, Viscardi G, et al. Induction of natural killer antibody-dependent cell cytotoxicity and of clinical activity of cetuximab plus avelumab in non-small cell lung cancer. ESMO Open 2020;5(5):e000753. https://doi.org/10.1136/esmoopen-2020-000753.
[43] Martinelli E, Martini G, Famiglietti V, Troiani T, Napolitano S, Pietrantonio F, et al. Cetuximab Rechallenge Plus Avelumab in Pretreated Patients With RAS Wild-type Metastatic Colorectal Cancer: The Phase 2 Single-Arm Clinical CAVE Trial. JAMA Oncol 2021;7(10):1529. https://doi.org/10.1001/jamaoncol.2021.2915.
[44] Ciardiello D, Famiglietti V, Napolitano S, Esposito L, Pietrantonio F, Avallone A, et al. Final results of the CAVE trial in RAS wild type metastatic colorectal cancer patients treated with cetuximab plus avelumab as rechallenge therapy: Neutrophil to lymphocyte ratio predicts survival. Clin Colorectal Cancer 2022. https://doi.org/10.1016/j.clcc.2022.01.005.
[45] Lonardi S, Prete AA, Morano F, Messina M, Formica V, Corsi DC, et al. Randomized phase II trial of avelumab alone or in combination with cetuximab for patients with previously treated, locally advanced, or metastatic squamous cell anal carcinoma: the CARACAS study. J Immunother Cancer 2021;9(11):e002996. https://doi.org/10.1136/jitc-2021-002996.
[46] Massarelli E, William W, Johnson F, Kies M, Ferrarotto R, Guo M, et al. Combining Immune Checkpoint Blockade and Tumor-Specific Vaccine for Patients With Incurable Human Papillomavirus 16-Related Cancer: A Phase 2 Clinical Trial. JAMA Oncol 2019;5(1):67. https://doi.org/10.1001/jamaoncol.2018.4051.
[47] Eng C, Fakih M, Amin M, Morris V, Hochster HS, Boland PM, et al. A phase II study of axalimogene filolisbac for patients with previously treated, unresectable, persistent/recurrent loco-regional or metastatic anal cancer. Oncotarget 2020;11(15):1334–43. https://doi.org/10.18632/oncotarget.27536.
[48] Mandriani B, Pelle' E, Pezzicoli G, Strosberg J, Abate-Daga D, Guarini A, et al. Adoptive T-cell immunotherapy in digestive tract malignancies: Current challenges and future perspectives. Cancer Treat Rev 2021;100:102288. https://doi.org/10.1016/j.ctrv.2021.102288.
[49] Stevanović S, Helman SR, Wunderlich JR, Langhan MM, Doran SL, Kwong MLM, et al. A Phase II Study of Tumor-infiltrating Lymphocyte Therapy for Human Papillomavirus-associated Epithelial Cancers. Clin Cancer Res 2019;25(5):1486–93. https://doi.org/10.1158/1078-0432.CCR-18-2722.
[50] Doran SL, Stevanović S, Adhikary S, Gartner JJ, Jia Li, Kwong MLM, et al. T-Cell Receptor Gene Therapy for Human Papillomavirus-Associated Epithelial Cancers: A First-in-Human. Phase I/II Study. J Clin Oncol 2019;37(30):2759–68. https://doi.org/10.1200/JCO.18.02424.
[51] Nagarsheth NB, Norberg SM, Sinkoe AL, Adhikary S, Meyer TJ, Lack JB, et al. TCR-engineered T cells targeting E7 for patients with metastatic HPV-associated epithelial cancers. Nat Med 2021;27(3):419–25. https://doi.org/10.1038/s41591-020-01225-1.
[52] Ravi R, Noonan KA, Pham V, Bedi R, Zhavoronkov A, Ozerov IV, et al. Bifunctional immune checkpoint-targeted antibody-ligand traps that simultaneously disable TGFβ enhance the efficacy of cancer immunotherapy. Nat Commun 2018;9(1). https://doi.org/10.1038/s41467-017-02696-6.
[53] Lan Y, Zhang D, Xu C, Hance KW, Marelli Bo, Qi J, et al. Enhanced preclinical antitumor activity of M7824, a bifunctional fusion protein simultaneously targeting PD-L1 and TGF-β. Sci Transl Med 2018;10(424). https://doi.org/10.1126/scitranslmed.aan5488.
[54] Ciardiello D, Elez E, Tabernero J, Seoane J. Clinical development of therapies targeting TGFβ: current knowledge and future perspectives. Ann Oncol 2020;31(10):1336–49. https://doi.org/10.1016/j.annonc.2020.07.00.
[55] O – Strauss J, Gatti-Mays M, Cho BC, et al. Long-term follow-up of patients with human papillomavirus (HPV)–associated malignancies treated with bintrafusp alfa, a bifunctional fusion protein targeting TGF-β and PD-L1. ESMO Congress 2021(16-21 September).
[56] Morris V, Liu S, Johnson B. Atezolizumab in combination with bevacizumab for patients with unresectable/metastatic anal cancer. Ann Oncol 2020;31(suppl_4):S409–61. https://doi.org/10.1016/annonc/annonc270.
[57] Shum E, Daud A, Reilley M, et al. 407 Preliminary safety, pharmacokinetics/pharmacodynamics, and antitumor activity of XmAb20717, a PD-1 x CTLA-4

bispecific antibody, in patients with advanced solid tumors. J ImmunoTher Cancer 2020:8. https://doi.org/10.1136/jitc-2020-SITC2020.0407.
[58] Martin D, Balermpas P, Gollrad J, Weiß C, Valentini C, Stuschke M, et al. RADIANCE - Radiochemotherapy with or without Durvalumab in the treatment of anal squamous cell carcinoma: A randomized multicenter phase II trial. Clin Transl Radiat Oncol 2020;23:43–9. https://doi.org/10.1016/j.ctro.2020.04.010.
[59] Hodi FS, Chiarion-Sileni V, Gonzalez R, Grob J-J, Rutkowski P, Cowey CL, et al. Nivolumab plus ipilimumab or nivolumab alone versus ipilimumab alone in advanced melanoma (CheckMate 067): 4-year outcomes of a multicentre, randomised, phase 3 trial. Lancet Oncol 2018;19(11):1480–92.
[60] Overman MJ, Lonardi S, Wong KYM, Lenz H-J, Gelsomino F, Aglietta M, et al. Durable Clinical Benefit With Nivolumab Plus Ipilimumab in DNA Mismatch Repair-Deficient/Microsatellite Instability-High Metastatic Colorectal Cancer. J Clin Oncol 2018;36(8):773–9. https://doi.org/10.1200/JCO.2017.76.9901.
[61] Kroemer G, Galluzzi L, Kepp O, Zitvogel L. Immunogenic cell death in cancer therapy. Annu Rev Immunol 2013;31(1):51–72. https://doi.org/10.1146/annurev-immunol-032712-100008.
[62] Apetoh L, Ladoire S, Coukos G, Ghiringhelli F. Combining immunotherapy and anticancer agents: the right path to achieve cancer cure? Ann Oncol 2015;26(9):1813–23. https://doi.org/10.1093/annonc/mdv209.
[63] Emens LA, Middleton G. The interplay of immunotherapy and chemotherapy: harnessing potential synergies. Cancer Immunol Res 2015;3(5):436–43. https://doi.org/10.1158/2326-6066.CIR-15-0064.
[64] Wahl AF, Donaldson KL, Faircnild C, Lee FYF, Foster SA, Demers GW, et al. Loss of normal p53 function confers sensitization to Taxol by increasing G2/M arrest and apoptosis. Nat Med 1996;2(1):72–9. https://doi.org/10.1038/nm0196-72.
[65] Kim S, Buecher B, André T, Jary M, Bidard F-C, Ghiringhelli F, et al. Atezolizumab plus modified docetaxel-cisplatin-5-fluorouracil (mDCF) regimen versus mDCF in patients with metastatic or unresectable locally advanced recurrent anal squamous cell carcinoma: a randomized, non-comparative phase II SCARCE GERCOR trial. BMC Cancer 2020;20(1). https://doi.org/10.1186/s12885-020-06841-1.
[66] Cushman TR, Gomez D, Kumar R, Likacheva A, Chang JY, Cadena AP, et al. Combining radiation plus immunotherapy to improve systemic immune response. J Thorac Dis 2018;10(S3):S468–79. https://doi.org/10.21037/jtd.2018.01.130.
[67] Kang J, Demaria S, Formenti S. Current clinical trials testing the combination of immunotherapy with radiotherapy. J Immunotherapy Cancer 2016;4(1). https://doi.org/10.1186/s40425-016-0156-7.
[68] Wen Lu, Tong F, Zhang R, Chen L, Huang Yu, Dong X. The Research Progress of PD-1/PD-L1 Inhibitors Enhancing Radiotherapy Efficacy. Front Oncol 2021;11. https://doi.org/10.3389/fonc.2021.799957.
[69] Ngwa W, Irabor OC, Schoenfeld JD, Hesser J, Demaria S, Formenti SC. Using immunotherapy to boost the abscopal effect. Nat Rev Cancer 2018;18(5):313–22. https://doi.org/10.1038/nrc.2018.6.
[70] Lopes A, Vandermeulen G, Préat V. Cancer DNA vaccines: current preclinical and clinical developments and future perspectives. J Exp Clin Cancer Res 2019;38(1). https://doi.org/10.1186/s13046-019-1154-7.