Anal Squamous Cell Carcinoma: From Standard Treatment to Personalized Therapy

Mustafa M. Basree, DO, MS;[1]* Ryan Hutten, MD;[1] Quaovi Sodji, MD, PhD;[1,2] Michael F. Bassetti, MD, PhD;[1] Jacob A. Miller, MD[3]

Abstract

Anal squamous cell carcinoma (ASCC) is a rare but increasingly prevalent disease, predominantly driven by human papillomavirus infection, with decreasing prevalence among individuals of vaccination-eligible age. In this review, we discuss both the current standard of care and future approaches for managing ASCC. There is interest in de-escalating therapy to minimize treatment-related morbidity, with studies such as DECREASE and PLATO currently ongoing. The integration of liquid biopsies as well as molecular biomarkers into clinical practice offers an exciting new frontier for personalized ASCC treatment. The future of anal cancer management lies in a personalized, biomarker-driven approach, which holds promise to transform clinical decision-making and enhance both the quantity and quality of life for patients with ASCC.

Keywords: anal cancer, anal squamous cell carcinoma, chemoradiation, virally mediated cancers, HPV-related anal cancer

Introduction

Anal squamous cell carcinoma (ASCC) is a relatively rare disease, accounting for roughly 0.5%[1] of all new cancer diagnoses in the United States (US). There is an annual percentage increase in new cases per year from 2.2 to 2.5 cases per 100,000 since the 1970s across racial categories.[1] Women are more likely to develop invasive carcinoma of the anus compared with men, with 7180 vs 3360 cases in the US in 2024, respectively.[2,3] Mortality estimates in 2024 are 2190 deaths, roughly equal between male and female patients.[2] The median age at diagnosis is 64 years, with a 1.65% annual increase in cases among patients aged 65 and older over the past decade and a 3.12% annual decrease in cases among patients younger than 50.[1]

A cross-sectional study of the US Cancer Statistics database showed that human papillomavirus (HPV) vaccination significantly reduced the incidence of ASCC among roughly 8000 vaccine-eligible patients aged 20-44 years.[3] The authors reported a 24% risk reduction (Relative Risk [RR], 0.76; 95% CI, 0.71-0.83) for in situ cases and 15% (RR, 0.85; 95% CI, 0.81-0.88) for invasive cases from 2009 to 2018 compared with 2001 to 2008.[3] Interestingly, rates of both in situ and invasive cases continue to rise in older, nonvaccination-eligible patients in the same period, highlighting the potential for prevention and early detection.[4] Despite the impact of

Affiliations: [1]Department of Human Oncology, University of Wisconsin Hospitals and Clinics, Madison, WI. [2]William S. Middleton Memorial Veterans Hospital, Madison, WI. [3]Department of Radiation Oncology, Cleveland Clinic, Cleveland, OH.
Corresponding author: *Mustafa M. Basree, DO, MS, Department of Human Oncology, University of Wisconsin Hospitals and Clinics, K4/438, 600 Highland Ave, Madison, WI 53792-3684. (mbasree@uwhealth.org)
Disclosure: The authors have no conflicts of interest to disclose. None of the authors received outside funding for the production of this original manuscript and no part of this article has been previously published elsewhere. The authors used ChatGPT version 4.0 for grammatical and stylistic edits after the manuscript was written.

Published: September 1, 2024. https://doi.org/10.37549/ARO-D-24-00026

HPV vaccination on the incidence of ASCC, vaccination rates remain low in the US, with only 38.6% of children aged 9-17 years having received at least 1 dose of the vaccine in 2022.[5]

This review will discuss the current standard of care for the management of ASCC and explore how advancements in molecular biomarkers are paving the way for personalized treatment strategies. Additionally, a summary of ongoing clinical trials in the context of those biomarkers will also be provided.

Risk Factors and Screening Considerations

Factors associated with ASCC include HPV infection (predominately genotypes 16 and 18), human immunodeficiency virus (HIV) positivity, sexually transmitted infections, immunosuppression, and tobacco use.[4,6] HPV status and p16 overexpression correlate with survival and recurrence outcomes.[7,8] In HPV-positive tumors, the dysfunction of p53 due to the HPV-E6 protein can sensitize tumors to chemoradiation (CRT). In non-HPV mediated anal cancers (10%-15% of cases), p53 suppression is often unrecoverable due to gene mutations,[7] lowering CRT effectiveness.[9,10] Non-HPV-16 genotypes are potentially more common among patients with HIV.[11]

Individuals with a history of HPV-mediated gynecologic cancers are at high risk for ASCC. The International Anal Neoplasia Society's (IANS) consensus guidelines recommend that women with a history of vulvar cancer or high-grade intraepithelial lesion (HSIL) to start screening for anal cancer within 1 year of diagnosis.[12] Screening for patients over 45 years old with a history of cervical or vaginal cancers or HSIL is determined on a case-by-case basis. A list of screening and diagnostic procedures is further reviewed in IANS consensus guidelines.[12] Furthermore, patients with a new diagnosis of anal intraepithelial lesion or ASCC are recommended to undergo screening for synchronous gynecologic malignancies or HSIL (cervical, vulvar, and/or vaginal) with a gynecologic examination including biopsy of suspicious lesions.[13] A Swedish population-based study of more than 3.7 million women showed an association between history of grade 3 cervical intraepithelial lesions (CIN) and the risk of developing anogenital cancers.[14] The risk of anal cancer was zero in the first year after a CIN diagnosis but increased yearly, with an incidence rate ratio of 4.98 after 10 years compared to women without a CIN diagnosis.[14] Therefore, physicians should remain vigilant in screening for anal cancer in patients with a prior history of gynecologic cancers.

Pretreatment evaluation involves a complete history, physical examination, digital rectal examination (DRE), inguinal nodal evaluation and, if applicable, a gynecologic examination. Staging involves CT scans of the chest, abdomen, and pelvis; pelvic MRI aids in anatomy delineation, treatment planning, and evaluating suspicious findings.[4,13] F-18 fluorodeoxyglucose positron emission tomography/CT (FDG-PET/CT) is recommended for nodal staging and metabolic activity of suspicious features on CT and/or MRI.[13]

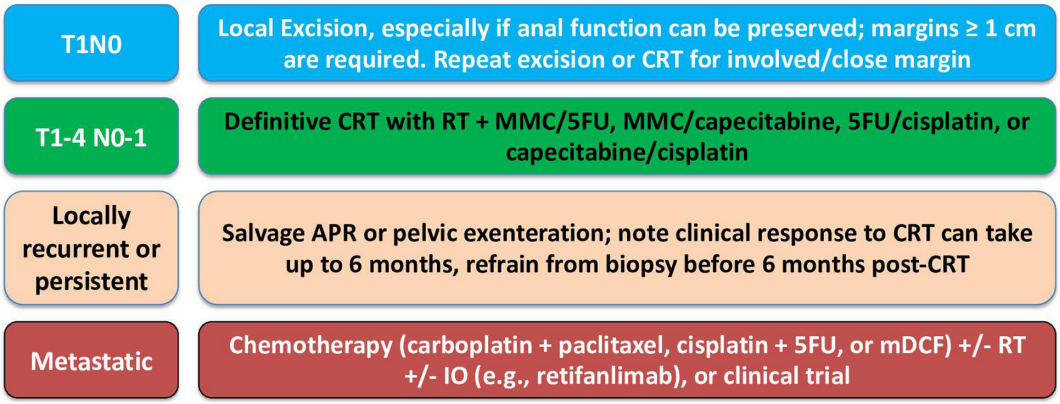
Standard-of-Care Management

Organ preservation is the standard of care for patients with nonmetastatic ASCC (**Figure 1**). For localized tumors less than or equal to 2 cm (T1 per American Joint Committee on Cancer 8th edn), local excision with at least a 1 cm margin may be considered if anal function can be preserved.[4,13] Local excision should generally be reserved for patients with an anal margin or peri-anal tumors with no or minimal involvement of anal sphincter complex. Involved or close margins warrant repeat excision, although this is often challenging. For cases in which excision is not feasible, definitive CRT with 5-fluorouracil (5FU) and either mitomycin C (MMC) or platinum is preferred.

Patients with locoregional disease (T1-4 N0-1 M0) are generally recommended definitive MMC/5FU-based CRT. Radiation alone[15-17] as well as MMC omission are associated[18] with inferior disease control (with better toxicity profiles) compared with CRT. Although MMC remains standard, replacement with cisplatin may achieve similar disease control and decreased hematological toxicity.[19] Capecitabine may be substituted for 5FU.[20-22] There is no role for induction[23,24] or maintenance[19] chemotherapy in nonmetastatic disease. Radiation doses are institution- and country-specific and range from 50 to 60 Gy to the primary tumor, 30.6 to 45 Gy to elective nodes, and 50 to 54 Gy to involved nodes.[4,13,19,24,25] Intensity-modulated radiation therapy (IMRT) is associated with lower toxicity profiles and fewer treatment breaks compared with 3D conformal radiation therapy.[22,25-27] Proton radiation therapy has not been shown to improve disease control,[28] toxicity profile[29] despite favorable dosimetry, or patient-reported outcomes compared with photon-based radiation therapy.[30]

Clinical response can continue up to 6 months post-CRT, even if a complete clinical response (cCR) is not observed by 3 months.[31] Biopsy before 6 months post-CRT is not recommended. The primary method

Figure 1. Clinical management



Abbreviations: 5FU, 5-fluorouracil; APR, abdominal peritoneal resection; CRT, chemoradiation; IO, immunotherapy; mDCF, modified docetaxel (40 mg/m^2), cisplatin (40 mg/m^2), and 5FU (1200 mg/m^2/day for 2 days), every 2 weeks intravenously; MMC, mitomycin C; RT, radiation therapy

of assessing treatment response is DRE and anoscopy, typically 26 weeks after CRT in line with the ACT-II study.[6] Post-treatment radiographic evaluation with pelvic MRI and/or FDG-PET/CT may also be utilized, although that is not routine. Surveillance for patients with cCR includes DRE and inguinal nodal examination every 3 to 6 months for 5 years, and anoscopy every 6 to 12 months for 3 years.[32] In patients with stage II-III disease, imaging of the chest, abdomen, and pelvis is completed annually for 3 years.[32] Approximately 80% of recurrences occur in the first 2 years post-CRT.[33] Up to a third of patients with persistent or recurrent disease will ultimately require salvage abdominal peritoneal resection or even pelvic exenteration depending on extent of disease.[4,13]

Patients with metastatic disease at diagnosis are recommended chemotherapy as first line with carboplatin + paclitaxel, cisplatin + 5 FU, or modified docetaxel + cisplatin + 5FU (mDCF).[4,13,32,34] The addition of checkpoint inhibition with chemotherapy as first-line treatment is institution dependent. While broadly speaking immunotherapy (IO) has been reserved as second-line treatment here, early readout from the PODIUM-303/InterAACT 2 study shows a modest PFS benefit (9.30 vs 7.39 months; $P = 0.0006$) with no difference in OS, although data is maturing.[35] Ongoing trials across the care continuum are summarized below. Five-year overall survival (OS) is 72% for all patients (76% for women; 64% for men), 86% and 39% for patients with localized and distant disease, respectively, with a trend for better outcomes among women.[1] Five-year overall and disease-free survival for those who underwent salvage surgery after CRT are lower, at approximately 40% to 50%.[4]

There does not appear to be a difference in OS between HIV-positive and HIV-negative ASCC patients.[36,37] One series reported higher local failures among HIV-positive patients, which are likely due to toxicity-related treatment breaks.[38] Importantly, low CD4 count (< 350 cells in one study[39] and < 200 in others[40,41]) and high viral load (> 700 copies/mL[39]) correlated with increased grade 3 or higher toxicity, treatment interruptions, and hospitalizations. Wexler and colleagues reported that patients with low CD4 count and high viral load had significantly worse 5-year overall- and cancer-specific survival.[39] Collectively, those studies underscore the importance of treating HIV in this patient population. Additionally, a review of 13 population-based HIV and cancer registries throughout the US with 24,486 patients (10.9% with HIV and 9.3% with AIDS) showed that HIV was associated with increased all-cause mortality (1.53, 95% CI, 1.42-1.64) and with increased anal cancer-specific mortality among female patients (1.52, 95% CI, 1.18-1.97).[42] The National Comprehensive Cancer Network provides a good review of the management of people living with HIV undergoing cancer treatment.[43]

Tailored Treatment Strategies

Reducing long-term toxicity without compromising cancer control is the focus of ongoing trials. The PersonaLising RadioTherapy dOse for Anal Cancer (PLATO; ISRCTN88455282) integrates ACT-3, ACT-4, and ACT-5 to tailor management using biology and margin data (**Table 1**).[44] For example, ACT-3 is a de-escalation phase 2 protocol that is evaluating observation in T1N0 patients with negative margins (no tumor on ink) post local excision while those

Table 1. Summary of Ongoing Clinical Trials in Anal Squamous Cell Carcinoma

TRIAL	PHASE	STUDY POPULATION	SAMPLE SIZE	STUDY ARMS	TARGET/ BIOMARKER	PRIMARY ENDPOINT
DECREASE (NCT04166318)	II, R	T1-2N0, M0	252	Standard vs lower-dose CRT	Clinical stage	2-year disease control; 1-year change in fecal incontinence Quality of life
PLATO[44] (ISRCTN88455282)						
ACT3	II, NR	T1N0 who underwent local excision	252	Observation or lower-dose CRT if close margin ≤1 mm)	Clinical stage	3-year locoregional failure
ACT4	II, R	T1-2 (< 4 cm) N0		Standard vs lower-dose CRT		
ACT5	Pilot/II/III, R	T3-4N0-3 or T2N1-3, M0		Standard vs higher-dose CRT		
CoRInTH[45] (NCT04046133)	Ib/II, NR	T3-4N+, M0	50	Pembrolizumab + CRT	PD-1/PD-L1	Safety and tolerability, up to 1 year
INTERACT-ION[46] (NCT04719988)	II, NR	T1-3N1 or T4N0, M0	55	Induction ezabenlimab + mDCF followed by consolidation ezabenlimab + mDCF + involved nodal radiation (if clinical response > 30%) or consolidation standard CRT (if < 30% response)	PD-1	10-month clinical complete response
TIRANUS[47] (NCT05661188)	II, NR	T1-4N0-1, M0	45	Atezolizumab + tiragolumab in combination with CRT	PD-L1/ TIGIT	26-week clinical complete response
ECOG-ACRIN EA2165[48] (NCT03233711)	III, R	T3-4N0 or T2-4N1, M0	344	CRT followed by nivolumab vs observation	PD-1	5-year DFS
NCI[49] (NCT04929028)	II, NR	T3-4N0 or T2-4N1 (high-risk), T1-2N0 (low-risk), M0, HIV+	53	Low-risk = reduced intensity CRT followed by observation High-risk = CRT followed by nivolumab	Clinical stage; HIV+	5-year incidence of grade 3-4 adverse events
SPARTANA[50] (NCT04894370)	II, NR	Metastatic	34	Immune stimulatory XRT (8 Gy to target lesions), followed by mDCF + spartalizumab, with consolidation multimodal treatment for residual disease (ablative treatment) Maintenance spartalizumab	PD-1	1-year PFS
ECOG-ACRIN EA2176[51] (NCT04444921)	III, R	Inoperable, recurrent, or metastatic	205	Carboplatin-paclitaxel followed by observation vs nivolumab	PD-1	2-year PFS
POD1UM-303/ InterAACT 2[52] (NCT04472429)	III, R	Inoperable, recurrent, or metastatic	308	Carboplatin-paclitaxel followed by observation vs retifanlimab	PD-1	4.5-year PFS

Abbreviations: ACT, UK anal cancer trial; CRT, chemoradiation; DCF, docetaxel (75 mg/m^2), cisplatin (75 mg/m^2), and 5-fluorouracil (750 mg/m^2/day for 5 days), every 3 weeks intravenously; DECREASE, De-Intensified ChemoRadiation for Early-Stage Anal Squamous Cell Carcinoma; DFS, disease-free survival; HIV, human immunodeficiency virus; mDCF, docetaxel (40 mg/m^2), cisplatin (40 mg/m^2), and 5-fluorouracil (1200 mg/m^2/day for 2 days), every 2 weeks intravenously; NCI, National Cancer Institute; NR, nonrandomized design; PD-1, programmed cell death-1; PD-L1, programmed cell death lignad-1; PFS, progression-free survival; PLATO, personalizing radiation therapy dose in anal cancer; R, randomized design; TIGIT, T cell immunoglobulin and ITIM domain.

with a close margin (≤ 1 mm) receive dose-reduced CRT (41.4 Gy/23 fractions).[44] Similarly, the ACT-4 study is evaluating dose-reduced CRT for patients with small tumors (T1-2N0 ≤4 cm). Early data show comparable 6-month cCR between dose-reduced (41.4 Gy/23 fractions) and standard (50.4 Gy/28 fractions) CRT, with lower toxicity in the dose-reduced arm.[53] These results support the feasibility of safely de-intensifying treatment in carefully selected patients. The ongoing DECREASE phase II trial[54] (ECOG-ACRIN 2182; NCT04166318) is evaluating de-escalated treatment for node-negative disease. T1N0 patients receive 36 Gy to the primary tumor and 32 Gy to elective nodes in 20 fractions, while T2N0 patients receive 41.4 Gy to the primary tumor and 34.5 Gy to elective nodes in 23 fractions. The standard arm delivers 50.4 Gy to the primary tumor and 42 Gy to elective nodes in 28 fractions. In addition, patients in the experimental arm receive a lower MMC dose (10 mg/m^2 vs 12 mg/m^2) and 1 less cycle of 5FU compared with the standard arm.

Conversely, treatment intensification is being explored for patients with more advanced disease, who are at higher risk of treatment failure. Secondary analysis of RTOG 9811 demonstrated poor 5-year disease-free survival (DFS) in patients with T3-4N+ disease ranging from 43% to 27%,[55] highlighting the need for more aggressive treatment. Within the PLATO framework, the ACT-5 trial intensifies radiation dose for high-risk patients (T3-4N0 or T2-4N+) up to 61.6 Gy in an effort to improve control. Moreover, a recent phase 3 study in Russia also evaluated adding paclitaxel to CRT in 144 ASCC patients (~72% N+; ~78% stage III).[56] The study was terminated prematurely in 2019 due to loss of access to mitomycin C. Within this limitation, paclitaxel appears to significantly improve 3-year DFS (87.1% vs 64.4%, $P = .001$) and OS (95.5% vs 80.0%, $P < .001$), with increased grade 3 to 4 toxicities (56.9% vs 26.4%, $P < .0001$), compared with CRT with doublet chemotherapy. This provides a signal for possible benefit of intensifying chemotherapy in this group of patients. Lastly, immunotherapy is being investigated in conjunction with chemotherapy for this higher risk population, which is reviewed later in this article.

While strides have been made in organ preservation and overall disease outcomes with CRT for patients with anal cancer, challenges remain in balancing long-term toxicity and treatment morbidity. There is a need to tailor these treatments to an individual patient's anatomic stage (as in DECREASE and PLATO) and also their molecular signatures, which provides a richer overview of each tumor's biology.

Emerging Biomarkers

Understanding the molecular interplay between HPV pathogenesis and genomic alterations is crucial for optimizing treatment outcomes and personalizing therapy. Patients with HPV-positive disease have better outcomes partly due to inherent HPV oncogenesis. PIK3CA mutations and PTEN loss are present in 30% and 14% of HPV-positive cases, respectively,[7] which was also observed in an exploratory whole-exome sequencing (WES) analysis of RTOG 9811 patients ($n = 62$).[57] In contrast, patients with HPV-independent disease are more likely to harbor higher p53 and CDKN2A mutation burdens, at 67% and 56%, respectively.[7] In 2010, Lampejo and colleagues reviewed multiple biomarkers and reported a potential prognostic value for p21, Bcl-2, NF-kB, and cyclin A.[58]

Exploratory analysis of RTOG 9811 noted additional mutations such as *FBXW7*, which were prevalent in 15% of the cohort and associated with worse disease-free survival (hazard ratio [HR] 2.47 [1.02-5.96], $P = .045$) and a signal for inferior OS (HR 2.61 [0.97-7.04], $P = .058$).[57] Aldersley and colleagues performed WES on 72 patients with anal cancer ($n = 56$ primary; $n = 31$ recurrent).[11] HPV integration was noted in 38% of cases and was more common in stages III-IV, at a rate of 2.69 integrations per sample compared with 0.91 integrations per sample for stages I-II ($P = .008$).[11] They were numerically more common in recurrent and metastatic disease than in primary disease (1.88 vs 1.10; $P = .092$). The integration events were often associated with copy number variations and amplifications of genes such as *PI3KCA*, *MYC*, and *CCND1*.[11] Interestingly, amplification of *TERT* and deletions of *ATR*, *FANCD2*, and *FHIT* were reliably more common in recurrent/metastatic vs primary tumors, with corresponding enrichment of DNA damage response gene in recurrent tumors.[11] The authors posit that enrichment of those genes in the context of recurrent deletions may contribute to tumor recurrence post CRT. It is conceivable that HPV integrants across human cancers take advantage of host genomic aberrations, increasing instability, and ultimately leading to tumorigenesis early on and treatment resistance later.[59,60] Since viral integration and genomic instability may worsen as infected cells progress to malignancy,[11,59,60] this provides a rationale for the prevention and aggressive treatment of premalignant lesions such as HSIL. The Anal Cancer-HSIL Outcomes Research (ANCHOR) study was a multi-institutional phase III study that sought to determine whether treating anal

HSIL reduces the risk of progression to anal cancer among HIV-positive patients compared with active surveillance.[61] Treatment included excision, ablation, or administration of topical agents. Active surveillance included high-resolution anoscopy at least every 6 months and an annual biopsy. In a cohort of roughly 4500 patients with a median follow-up of 25.8 months, risk of progression to invasive disease was reduced by 57% (95% CI, 6-80; $P = .03$) among those who received treatment.[61]

Liquid biopsies, including circulating tumor cells and circulating tumor DNA (ctDNA), have emerged as an adjunct in identifying genomic alterations and monitoring treatment response across various cancers, including gastrointestinal cancer. In the noncomparative phase II study SCARCE C17-02 PRODIGE 60, combining IO with chemotherapy, patients with complete molecular response (cMR) as measured by HPV ctDNA pre- and post-treatment had better 1-year progression-free survival (PFS) (60.4% vs 15.4%) and OS (90.7% vs 64.2%) compared with those without cMR, respectively.[62] Moreover, Epitopes-HPV02 was a phase II single-arm study (NCT02402842) of patients with unresectable locally advanced/recurrent or metastatic ASCC where HPV ctDNA was evaluated as a predictive biomarker.[63] Positive HPV ctDNA at baseline did not correlate with PFS, although patients with a baseline ctDNA level < 2940 copies/mL had better PFS (HR, 2.1; 95% CI, 1.0-4.2; $P = .04$).[63] Like the SCARCE C17-02 study, cMR was associated with better 1-year OS (87% vs 50%) with an odds ratio of 7 (95% CI, 1.5-28.5; $P = .02$).[63] This is an exciting area of care in anal cancer; the use of blood biomarkers to guide clinical decision-making is under study in other virally[64] and nonvirally[65-68] mediated cancers. While prospective studies are underway evaluating its role in guiding management,[69,70] data show that HPV ctDNA in anal cancer may indeed be an important prognosticator.

Furthermore, tumor-infiltrating lymphocytes (TILs) have been suggested to correlate with outcomes in patients with HPV-mediated disease, supporting a role for the way the adaptive immune system behaves in virally mediated cancers.[9,71] Patients with high TILs had significantly longer disease-free intervals compared with patients with absent/low TILs (92% vs 63 %; log-rank $P = .006$),[71] in line with other HPV-mediated cancers.[72-76]

The identification and integration of these biomarkers into clinical practice have expanded our understanding of ASCC, offering new avenues for targeted therapies. Immunotherapy has gained significant interest recently as a promising treatment for ASCC as it revolutionized the field of oncology with its success across many different cancers.

The Promise of Immunotherapy

HPV inherently furnishes an immunosuppressive and evasive environment through multiple mechanisms, one of which is upregulation of programmed death ligand-1 (PD-L1).[77-79] While this provided a rationale to try different IO agents, outcomes in ASCC have been suboptimal.[80] Results of the randomized noncomparative phase II study SCARCE C17-02 PRODIGE 60 were recently published.[62] In nonbiomarker selected, chemo-naïve, patients with locally advanced or metastatic ASCC, the addition of atezolizumab to mDCF vs mDCF alone did not meet the primary endpoint of 1-year PFS (45% vs 43%). The combination of mDCF + IO was associated with higher grades 3-4 (61% vs 42%) and serious adverse events (25% vs 12%).[62] Interestingly, in patients with a PD-L1 combined positive score (CPS) of ≥ 5% ($n = 10$), 1-year PFS with atezolizumab + mDCF was 70% (95% CI, 47-100) compared with 39% (24-62) in the CPS-negative group ($n = 28$). This is in line with other studies showing IO responders are more likely to have higher PD-1/PD-L1 levels,[81-83] albeit low response overall.

The INTERACT-ION is another phase II study from the French group that is studying the role of induction ezabenlimab, an anti-PD-1 antibody, in combination with mDCF as an induction regimen before CRT in treatment-naïve patients with locally advanced, stage III (T4N0 or TxN+), ASCC, with promising early results.[46] Moreover, dual checkpoint inhibition is of interest in ASCC as it has been shown to be more efficacious in activating the immune system.[84-87] T cell immunoglobulin and ITIM domain (TIGIT) is an immune checkpoint receptor constitutively expressed on Tregs and is critical in mediating immunosuppression.[88] TIRANUS out of Spain is a single-arm phase II study (NCT05201612) that is studying the co-inhibition of PD-L1/TIGIT with atezolizumab and tiragolumab in combination with CRT for nonmetastatic patients with ASCC.[47]

Several trials of IO are underway across the continuum of ASCC care. For instance, pembrolizumab is currently part of the single-arm phase Ib/II CoRInTH trial (NCT04046133) combining the PD-1 agent with CRT in locally advanced stage III-IV ASCC.[45] The National Cancer Institute (NCI) has 2 phase II studies with nivolumab following definitive CRT. The first study is in high-risk stage II-IIIB patients (EA2165; NCT03233711)[48] with primary endpoint of PFS. The second study is a risk-adapted trial (NCT04929028) of either nivolumab (high-risk, T3-4N0M0 or T2-4N1M0) or observation

(low-risk, T1-2N0M0 or tumors <4 cm) following CRT in HIV-positive patients.[49] In more advanced disease, SPARTANA (NCT04894370) is a unique phase IIA study in metastatic ASCC that leverages radiation synergistic priming of the immune response (single-fraction 8 Gy to a target lesion) before starting spartalizumab (PD-1 inhibitor) and mDCF.[50] This regimen is then followed by consolidative ablative treatment to residual disease and maintenance spartalizumab, with the primary end point of PFS. Nivolumab (EA2176; NCT04444921)[51] and retifanlimab (PODIUM-303/InterAACT 2; NCT04472429)[52] are also being investigated in a phase III randomized fashion in combination with carboplatin/paclitaxel in metastatic and locally advanced/metastatic disease, respectively. As noted above, early data from the PODIUM-303/InterAACT 2 study show a signal of efficacy in terms of PFS but not OS at this time, with data continuing to mature. A summary of ongoing clinical trials is provided in **Table 1**.

While studies are ongoing, IO holds great potential as an adjunct to standard-of-care management in all stages of the disease. However, nonbiomarker-driven IO studies may prove futile, underscoring the importance of personalizing therapy.

Conclusion

The future of anal cancer management has the potential to provide personalized treatment and follow-up, moving away from a one-size-fits-all approach. This hope is derived from advancements in molecular and genomic profiling. The integration of emerging biomarkers such as HPV DNA and PD-L1 expression, along with disease staging, into clinical practice allows for tailored treatment strategies. This can improve patient outcomes and reduce treatment-related morbidity. As our understanding of the molecular underpinnings of ASCC deepens, this approach has the potential to transform care and improve both the quantity and quality of life for patients with ASCC.

References

1) Anus, Anal Canal & Anorectum. Long-Term Trends in SEER Age-Adjusted Incidence Rates, 1975-2021. NIH National Cancer Institute. Surveillance, Epidemiology, and End Results Program. Accessed July 15, 2024. https://seer.cancer.gov/statistics-network/explorer/application.html

2) Key statistics for anal cancer. Accessed July 15, 2024. https://www.cancer.org/cancer/types/anal-cancer/about/what-is-key-statistics.html

3) Berenson AB, Guo F, Chang M. Association of human papillomavirus vaccination with the incidence of squamous cell carcinomas of the anus in the US. *JAMA Oncol*. 2022;8(4):1-3. doi:10.1001/jamaoncol.2021.7652

4) Eng C, Ciombor KK, Cho M, et al. Anal cancer: emerging standards in a rare disease. *J Clin Oncol*. 2022;40(24):2774-2788. doi:10.1200/JCO.21.02566

5) Villarroel MA, Galinksy AM, Lu PJ, Pingali C. Human papillomavirus vaccination coverage in children ages 9-17 Years: United States; 2022. Accessed July 18, 2024. https://stacks.cdc.gov/view/cdc/145593

6) Eng C, Messick C, Glynne-Jones R. The management and prevention of anal squamous cell carcinoma. *Am Soc Clin Oncol Educ Book*. 2019;39(39):216-225. doi:10.1200/EDBK_237433

7) 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):156-162. doi:10.1038/bjc.2016.398

8) Serup-Hansen E, Linnemann D, Skovrider-Ruminski W, et al. 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):1812-1817. doi:10.1200/JCO.2013.52.3464

9) Bruyere D, Monnien F, Colpart P, et al. Treatment algorithm and prognostic factors for patients with stage I-III carcinoma of the anal canal: a 20-year multicenter study. *Mod Pathol*. 2021;34(1):116-130. doi:10.1038/s41379-020-0637-6

10) Meulendijks D, Tomasoa NB, Dewit L, 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):1358-1366. doi:10.1038/bjc.2015.20

11) Aldersley J, Lorenz DR, Mouw KW, D'Andrea AD, Gabuzda D. Genomic landscape of primary and recurrent anal squamous cell carcinomas in relation to HPV integration, copy-number variation, and DNA damage response genes. *Mol Cancer Res*. 2021;19(8):1308-1321. doi:10.1158/1541-7786.MCR-20-0884

12) Stier EA, Clarke MA, Deshmukh AA, et al. International anal neoplasia society's consensus guidelines for anal cancer screening. *Int J Cancer*. 2024;154(10):1694-1702. doi:10.1002/ijc.34850

13) Rao S, Guren MG, Khan K, et al. Anal cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up . *Ann Oncol*. 2021;32(9):1087-1100. doi:10.1016/j.annonc.2021.06.015

14) Edgren G, Sparén P. Risk of anogenital cancer after diagnosis of cervical intraepithelial neoplasia: a prospective population-based study. *Lancet Oncol*. 2007;8(4):311-316. doi:10.1016/S1470-2045(07)70043-8

15) Northover J, Glynne-Jones R, Sebag-Montefiore D, et al. Chemoradiation for the treatment of epidermoid anal cancer: 13-year follow-up of the first randomised UKCCCR Anal Cancer Trial (ACT I). *Br J Cancer*. 2010;102(7):1123-1128. doi:10.1038/sj.bjc.6605605

16) Epidermoid anal cancer: results from the UKCCCR randomised trial of radiotherapy alone versus radiotherapy, 5-fluorouracil, and mitomycin. *Lancet*. 1996;348(9034):1049-1054. doi:10.1016/S0140-6736(96)03409-5

17) Bartelink H, Roelofsen F, Eschwege F, et al. Concomitant radiotherapy and chemotherapy is superior to radiotherapy alone in the treatment of locally advanced anal cancer: results of a phase III randomized trial of the European Organization for Research and Treatment of Cancer radiotherapy and gastrointestinal cooperative groups. *J Clin Oncol*. 1997;15(5):2040-2049. doi:10.1200/JCO.1997.15.5.2040

18) Flam M, John M, Pajak TF, et al. Role of mitomycin in combination with fluorouracil and radiotherapy, and of salvage chemoradiation in the definitive nonsurgical treatment of epidermoid carcinoma of the anal canal: results of a phase III randomized intergroup study. *J Clin Oncol*. 1996;14(9):2527-2539. doi:10.1200/JCO.1996.14.9.2527

19) James RD, Glynne-Jones R, Meadows HM. Mitomycin or cisplatin chemoradiation with or without maintenance chemotherapy for treatment of squamous-cell carcinoma of the anus (ACT II): a randomised, phase 3, open-label, 2 × 2 factorial trial. *Lancet Oncol*. 2013;14(6):516-524. doi:10.1016/S1470-2045(13)70086-X

20) Goodman KA, Julie D, Cercek A, et al. Capecitabine with mitomycin reduces acute hematologic toxicity and treatment delays in patients undergoing definitive chemoradiation using intensity modulated radiation therapy for anal cancer. *Int J Radiat Oncol Biol Phys*. 2017;98(5):1087-1095. doi:10.1016/j.ijrobp.2017.03.022

21) Glynne-Jones R, Meadows H, Wan S, et al. Extra—a multicenter phase II study of chemoradiation using a 5 day per week oral regimen of capecitabine and intravenous mitomycin C in anal cancer. *Int J Radiat Oncol Biol Phys*. 2008;72(1):119-126. doi:10.1016/j.ijrobp.2007.12.012

22) Gilbert A, Drinkwater K, McParland L. UK national cohort of anal cancer treated with intensity-modulated radiotherapy: one-year oncological and patient-reported outcomes. *Eur J Cancer*. 2020;128:7-16. doi:10.1016/j.ejca.2019.12.022

23) Gunderson LL, Winter KA, Ajani JA, et al. Long-term update of US GI intergroup RTOG 98-11 phase III trial for anal carcinoma: survival, relapse, and colostomy failure with concurrent chemoradiation involving fluorouracil/mitomycin versus fluorouracil/cisplatin. *J Clin Oncol*. 2012;30(35):4344-4351. doi:10.1200/JCO.2012.43.8085

24) Peiffert D, Tournier-Rangeard L, Gérard J-P, et al. Induction chemotherapy and dose intensification of the radiation boost in locally advanced anal canal carcinoma: final analysis of the randomized UNICANCER ACCORD 03 trial. *J Clin Oncol*. 2012;30(16):1941-1948. doi:10.1200/JCO.2011.35.4837

25) Kachnic LA, Winter K, Myerson RJ, et al. RTOG 0529: a phase 2 evaluation of dose-painted intensity modulated radiation therapy in combination with 5-fluorouracil and mitomycin-C for the reduction of acute morbidity in carcinoma of the anal canal. *Int J Radiat Oncol Biol Phys*. 2013;86(1):27-33. doi:10.1016/j.ijrobp.2012.09.023

26) Bryant AK, Huynh-Le M-P, Simpson DR, et al. Intensity modulated radiation therapy versus conventional radiation for anal cancer in the veterans affairs system. *Int J Radiat Oncol Biol Phys*. 2018;102(1):109-115. doi:10.1016/j.ijrobp.2018.05.044

27) Han K, Cummings BJ, Lindsay P, et al. Prospective evaluation of acute toxicity and quality of life after IMRT and concurrent chemotherapy for anal canal and perianal cancer. *Int J Radiat Oncol Biol Phys*. 2014;90(3):587-594. doi:10.1016/j.ijrobp.2014.06.061

28) Lee G, Nelson B, Koenig JL, et al. Long-term outcomes of definitive chemoradiation with proton therapy for treatment of carcinoma of the anal canal: combined analysis of two prospective trials. *International Journal of Radiation Oncology Biology Physics*. 2024;120(2):S183-S184. doi:10.1016/j.ijrobp.2024.07.2228

29) Mohiuddin JJ, Jethwa KR, Grandhi N, et al. Multi-institutional comparison of intensity modulated photon versus proton radiation therapy in the management of squamous cell carcinoma of the anus. *Adv Radiat Oncol*. 2021;6(5):100744. doi:10.1016/j.adro.2021.100744

30) Shuja M, Shah SN, Harmsen WS, et al. Patient reported outcomes after chemoradiation with IMPT vs. IMRT for patients with anal canal squamous cell carcinoma. *Int J Radiat Oncol Biol Phys*. 2024;120(2):S184. doi:10.1016/j.ijrobp.2024.07.2229

31) Glynne-Jones R, Sebag-Montefiore D, Meadows HM, et al. Best time to assess complete clinical response after chemoradiotherapy in squamous cell carcinoma of the anus (ACT II): a post-hoc analysis of randomised controlled phase 3 trial. *Lancet Oncol*. 2017;18(3):347-356. doi:10.1016/S1470-2045(17)30071-2

32) NCCN Guidelines. Anal Carcinoma. NCCN. Accessed July 18, 2024. https://www.nccn.org/guidelines/guidelines-detail?category=1&id=1414

33) Frazer ML, Yang G, Felder S, et al. Determining optimal follow-up for patients with anal cancer following chemoradiation. *Am J Clin Oncol*. 2020;43(5):319-324. doi:10.1097/COC.0000000000000673

34) Rao S, Sclafani F, Eng C, 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):2510-2518. doi:10.1200/JCO.19.03266

35) Rao S, Samalin-Scalzi E, Evesque L, et al. LBA2 POD1UM-303/interaact 2: phase III study of retifanlimab with carboplatin-paclitaxel (c-p) in patients (pts) with inoperable locally recurrent or metastatic squamous cell carcinoma of the anal canal (SCAC) not previously treated with systemic chemotherapy (chemo). *Annals of Oncology*. 2024;35:S1217. doi:10.1016/j.annonc.2024.08.2262

36) White EC, Khodayari B, Erickson KT, et al. Comparison of toxicity and treatment outcomes in HIV-positive versus HIV-negative patients with squamous cell carcinoma of the anal canal. *Am J Clin Oncol*. 2017;40(4):386-392. doi:10.1097/COC.0000000000000172

37) Fraunholz I, Weiss C, Eberlein K, Haberl A, Rödel C. Concurrent chemoradiotherapy with 5-fluorouracil and mitomycin C for invasive anal carcinoma in human immunodeficiency virus-positive patients receiving highly active antiretroviral therapy. *Int J Radiat Oncol Biol Phys*. 2010;76(5):1425-1432. doi:10.1016/j.ijrobp.2009.03.060

38) Oehler-Jänne C, Huguet F, Provencher S, et al. HIV-specific differences in outcome of squamous cell carcinoma of the anal canal: a multicentric cohort study of HIV-positive patients receiving highly active antiretroviral therapy. *J Clin Oncol*. 2008;26(15):2550-2557. doi:10.1200/JCO.2007.15.2348

39) Wexler A, Berson AM, Goldstone SE, et al. Invasive anal squamous-cell carcinoma in the HIV-positive patient: outcome in the era of highly active antiretroviral therapy. *Dis Colon Rectum*. 2008;51(1):73-81. doi:10.1007/s10350-007-9154-7

40) Edelman S, Johnstone PAS. Combined modality therapy for HIV-infected patients with squamous cell carcinoma of the anus: outcomes and toxicities. *Int J Radiat Oncol Biol Phys*. 2006;66(1):206-211. doi:10.1016/j.ijrobp.2006.03.049

41) Hoffman R, Welton ML, Klencke B, Weinberg V, Krieg R. The significance of pretreatment CD4 count on the outcome and treatment tolerance of HIV-positive patients with anal cancer. *Int J Radiat Oncol Biol Phys*. 1999;44(1):127-131. doi:10.1016/s0360-3016(98)00528-8

42) Shing JZ, Engels EA, Austin AA. Survival by sex and HIV status in patients with anal cancer in the USA between 2001 and 2019: a retrospective cohort study. *Lancet HIV*. 2024;11(1):e31-e41. doi:10.1016/S2352-3018(23)00257-6

43) NCCN Guidelines. Cancer in People with HIV, NCCN. Accessed August 8, 2024. https://www.nccn.org/guidelines/guidelines-detail?category=4&id=1487

44) PLATO - Personalising anal cancer radiotherapy dose. Health Research Authority. Accessed July 19, 2024. https://www.hra.nhs.uk/planning-and-improving-research/application-summaries/research-summaries/plato-personalising-anal-cancer-radiotherapy-dose/

45) Hall M, Adams R, Guren MG, et al. CoRInTH: a phase ib/II trial of checkpoint inhibitor, pembrolizumab (PD-1 antibody [ab]) plus standard Intensity Modulated Chemoradiotherapy (IMCRT) in HPV-induced stage III Squamous Cell Carcinoma (SCC) of the anus. *J Clin Oncol*. 2022;40(16_suppl):TPS3630-TPS3630. doi:10.1200/JCO.2022.40.16_suppl.TPS3630

46) Kim S, Boustani J, Vernerey D, et al. Ezabenlimab (BI 754091) plus modified docetaxel, cisplatin, and 5-fluorouracil (mdcf) followed by chemoradiotherapy (CRT) in patients (pts) with stage III squamous cell anal carcinoma (SCCA): early efficacy endpoint results from the phase II INTERACT-ION study. *J Clin Oncol*. 2024;42(3_suppl):2-2. doi:10.1200/JCO.2024.42.3_suppl.2

47) Capdevila J, Guillot M, Pampols M, et al. Atezolizumab plus tiragolumab in combination with chemoradiotherapy in localized squamous cell carcinoma of the anal canal: TIRANUS (GEMCAD-2103) trial. *J Clin Oncol*. 2023;41(16_suppl):TPS3624-TPS3624. doi:10.1200/JCO.2023.41.16_suppl.TPS3624

48) Nivolumab after combined modality therapy in treating patients with high risk stage II-IIIB anal cancer. NIH National Library of Medicine. Accessed July 19, 2024. https://clinicaltrials.gov/study/NCT03233711

49) Therapy adapted for high risk and low risk HIV-associated anal cancer. NIH National Library of Medicine. Accessed July 21, 2024. https://clinicaltrials.gov/study/NCT04929028#study-plan

50) Combination of Spartalizumab, mDCF and Radiotherapy in patients with metastatic squamous cell anal carcinoma. NIH National Library of Medicine. Accessed July 21, 2024. https://clinicaltrials.gov/study/NCT04894370

51) EA2176: Phase 3 Clinical trial of carboplatin and paclitaxel +/- nivolumab in metastatic anal cancer patients. NIH National Library of Medicine. Accessed July 21, 2024. https://clinicaltrials.gov/study/NCT04444921

52) Rao S, Jones M, Bowman J, Tian C, Spano JP. POD1UM-303/interaact 2: a phase III, global, randomized, double-blind study of retifanlimab or placebo plus carboplatin-paclitaxel in patients with locally advanced or metastatic squamous cell anal carcinoma. *Front Oncol*. 2022;12:935383. doi:10.3389/fonc.2022.935383

53) Gilbert A, et al. Standard vs dose reduced chemoradiotherapy in anal cancer: short-term results of the PLATO-ACT4 RCT. Accessed October 15, 2024. https://www.estro.org/Congresses/ESTRO-2023/1438/highlightsofprofferedpapers-latestclinicaltrials/13339/standardvsdosereducedchemoradiotherapyinanalcancer

54) Lower-dose chemoradiation in treating patients with early-stage anal cancer, the decrease study. NIH National Library of Medicine. Accessed July 19, 2024. https://clinicaltrials.gov/study/NCT04166318

55) Gunderson LL, Moughan J, Ajani JA, et al. Anal carcinoma: impact of TN category of disease on survival, disease relapse, and colostomy failure in US gastrointestinal intergroup RTOG 98-11 phase 3 trial. *Int J Radiat Oncol Biol Phys*. 2013;87(4):638-645. doi:10.1016/j.ijrobp.2013.07.035

56) Gordeev SS, Naguslaeva AA, Chernykh MV, et al. The addition of paclitaxel in chemoradiotherapy of anal squamous cell carcinoma: a prospective randomized phase 3 trial. *Koloproktologiâ*. 2022;21(4):30-38. doi:10.33878/2073-7556-2022-21-4-30-38

57) Konieczkowski DJ, Rawal B, Reardon B, et al. The genomic landscape of anal cancer and its relationship to clinical outcomes: an exploratory analysis of NRG oncology/RTOG 98-11. *Int J Radiat Oncol Biol Phys*. 2019;105(1):S104-S105. doi:10.1016/j.ijrobp.2019.06.594

58) Lampejo T, Kavanagh D, Clark J, et al. Prognostic biomarkers in squamous cell carcinoma of the anus: a systematic review. *Br J Cancer*. 2010;103(12):1858-1869. doi:10.1038/sj.bjc.6605984

59) Akagi K, Li J, Broutian TR, et al. Genome-wide analysis of HPV integration in human cancers reveals recurrent, focal genomic instability. *Genome Res*. 2014;24(2):185-199. doi:10.1101/gr.164806.113

60) Bodelon C, Vinokurova S, Sampson JN, et al. Chromosomal copy number alterations and HPV integration in cervical precancer and invasive cancer. *Carcinogenesis*. 2016;37(2):188-196. doi:10.1093/carcin/bgv171

61) Palefsky JM, Lee JY, Jay N, et al. Treatment of anal high-grade squamous intraepithelial lesions to prevent anal cancer. *N Engl J Med*. 2022;386(24):2273-2282. doi:10.1056/NEJMoa2201048

62) Kim S, Ghiringhelli F, de la Fouchardière C, et al. Atezolizumab plus modified docetaxel, cisplatin, and fluorouracil as first-line treatment for advanced anal cancer (SCARCE C17-02 PRODIGE 60): a randomised, non-comparative, phase 2 study. *Lancet Oncol*. 2024;25(4):518-528. doi:10.1016/S1470-2045(24)00081-0

63) Bernard-Tessier A, Jeannot E, Guenat D, et al. Clinical validity of HPV circulating tumor DNA in advanced anal carcinoma: an ancillary study to the epitopes-HPV02 trial. *Clinical Cancer Research*. 2019;25(7):2109-2115. doi:10.1158/1078-0432.CCR-18-2984

64) Detecting HPV DNA in anal and cervical cancers. NIH National Library of Medicine. Accessed July 20, 2024. https://clinicaltrials.gov/study/NCT04857528

65) NRG-GI008 CIRCULATE-US. NRG. Accessed July 20, 2024. http://www.nrgoncology.org/Clinical-Trials/Protocol/nrg-gi008

66) Nors J, Henriksen TV, Gotschalck KA, et al. IMPROVE-IT2: implementing noninvasive circulating tumor DNA analysis to optimize the operative and postoperative treatment for patients with colorectal cancer - intervention trial 2. study protocol. *Acta Oncol*. 2020;59(3):336-341. doi:10.1080/0284186X.2019.1711170

67) Individualized treatment in treating patients with stage II-IVB Nasopharyngeal cancer based on EBV DNA. NIH National Library of Medicine. Accessed July 20, 2024. https://clinicaltrials.gov/study/NCT02135042

68) Human Papilloma Virus (HPV) Circulating Tumor DNA (ctDNA) in cervical cancer. NIH National Library of Medicine. Accessed July 20, 2024. https://clinicaltrials.gov/study/NCT04574635

69) NOAC9 - Circulating Tumor DNA Guided Follow-Up in Anal Cancer. NIH National Library of Medicine. Accessed July 20, 2024. https://clinicaltrials.gov/study/NCT05572801

70) Circulating biomarkers in patients with anal cancer treated with induction chemotherapy. NIH National Library of Medicine. Accessed July 21, 2024. https://clinicaltrials.gov/study/NCT05233540

71) Gilbert DC, Serup-Hansen E, Linnemann D, et al. Tumour-infiltrating lymphocyte scores effectively stratify outcomes over and above p16 post chemo-radiotherapy in anal cancer. *Br J Cancer*. 2016;114(2):134-137. doi:10.1038/bjc.2015.448

72) Litwin TR, Irvin SR, Chornock RL, et al. Infiltrating T-cell markers in cervical carcinogenesis: a systematic review and meta-analysis. *Br J Cancer*. 2021;124(4):831-841. doi:10.1038/s41416-020-01184-x

73) Gies S, Melchior P, Stroeder R, et al. Immune landscape of vulvar cancer patients treated with surgery and adjuvant radiotherapy revealed restricted T cell functionality and increased IL-17 expression associated with cancer relapse. *Int J Cancer*. 2024;154(2):343-358. doi:10.1002/ijc.34745

74) Kortekaas KE, Santegoets SJ, Tas L, et al. Primary vulvar squamous cell carcinomas with high T cell infiltration and active immune signaling are potential candidates for neoadjuvant PD-1/PD-L1 immunotherapy. *J Immunother Cancer*. 2021;9(10):e003671. doi:10.1136/jitc-2021-003671

75) Spector ME, Bellile E, Amlani L, et al. Prognostic value of tumor-infiltrating lymphocytes in head and neck squamous cell carcinoma. *JAMA Otolaryngol Head Neck Surg*. 2019;145(11):1012. doi:10.1001/jamaoto.2019.2427

76) Ward MJ, Thirdborough SM, Mellows T, et al. Tumour-infiltrating lymphocytes predict for outcome in HPV-positive oropharyngeal cancer. *Br J Cancer*. 2014;110(2):489-500. doi:10.1038/bjc.2013.639

77) Liu C, Lu J, Tian H, et al. Increased expression of PD-L1 by the human papillomavirus 16 E7 oncoprotein inhibits anticancer immunity. *Mol Med Rep*. 2017;15(3):1063-1070. doi:10.3892/mmr.2017.6102

78) Huffman BM, Singh H, Ali LR. Biomarkers of pembrolizumab efficacy in advanced anal squamous cell carcinoma: analysis of a phase II clinical trial and a cohort of long-term responders. *J Immunother Cancer*. 2024;12(1):e008436. doi: 10.1136/jitc-2023-008436

79) Koneva LA, Zhang Y, Virani S, et al. HPV integration in HNSCC correlates with survival outcomes, immune response signatures, and candidate drivers. *Mol Cancer Res*. 2018;16(1):90-102. doi:10.1158/1541-7786.MCR-17-0153

80) Martinelli E, Ciardiello D. Combining chemotherapy and immunotherapy for advanced anal cancer: Are we ready? *Lancet Oncol*. 2024;25(4):416-417. doi:10.1016/S1470-2045(24)00131-1

81) Morris VK, Salem ME, Nimeiri H. Nivolumab for previously treated unresectable metastatic anal cancer (NCI9673): a multicentre, single-arm, phase 2 study. *Lancet Oncol*. 2017;18(4):446-453. doi: 10.1016/S1470-2045(17)30104-3

82) Prete AA, Manca P, Messina M, et al. Extensive molecular profiling of squamous cell anal carcinoma in a phase 2 trial population: translational analyses of the "CARACAS" study. *Eur J Cancer*. 2023;182:87-97. doi:10.1016/j.ejca.2022.12.025

83) Marabelle A, Cassier PA, Fakih M, 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;7(5):446-454. doi:10.1016/S2468-1253(21)00382-4

84) Cho BC, Abreu DR, Hussein M, et al. Tiragolumab plus atezolizumab versus placebo plus atezolizumab as a first-line treatment for PD-L1-selected non-small-cell lung cancer (CITYSCAPE): primary and follow-up analyses of a randomised, double-blind, phase 2 study. *Lancet Oncol*. 2022;23(6):781-792. doi:10.1016/S1470-2045(22)00226-1

85) Ilhan-Mutlu A. Dual immune checkpoint blockade in gastroesophageal tumors: never say never. Memo - Mag Eur Med Oncol. 2023;16(1):56-61. doi:10.1007/s12254-022-00870-1

86) O'Malley DM, Neffa M, Monk BJ, et al. Dual PD-1 and CTLA-4 checkpoint blockade using balstilimab and zalifrelimab combination as second-line treatment for advanced cervical cancer: an open-label phase II study. *JCO*. 2022;40(7):762-771. doi: 10.1200/JCO.21.02067

87) Frentzas S, Kao S, Gao R, et al. AdvanTIG-105: a phase I dose escalation study of the anti-TIGIT monoclonal antibody ociperlimab in combination with tislelizumab in patients with advanced solid tumors. *J Immunother Cancer*. 2023;11(10):e005829. doi:10.1136/jitc-2022-005829

88) Chu X, Tian W, Wang Z, Zhang J, Zhou R. Co-inhibition of TIGIT and PD-1/PD-L1 in cancer immunotherapy: mechanisms and clinical trials. *Mol Cancer*. 2023;22(1):93. doi: 10.1186/s12943-023-01800-3