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A 2020 Update of anal cancer: The increasing problem in women and expanding treatment landscape

Caroline Lum[1], Hans Prenen[1,2], Amy Body[1], Marissa Lam[1], Eva Segelov[1,3*]

[a]Medical Oncology, Monash Medical Centre, Clayton, Australia; [b]Oncology Department, University Hospital Antwerp, Antwerp, Belgium; [c]Monash University, Clayton, Australia

* Correspondence:

Professor Eva Segelov

Lvl 7, MHTP building, Monash Health 246 Clayton Rd Clayton VIC 3168 Australia

E: eva.segelov@monashhealth.org

Ph: 61 3 85722392
F: 61 3 85722446

Abstract

Introduction: Anal cancer is a rare malignancy with increasing incidence, notably in women. This disease is highly associated with HPV infection and its incidence and mortality are currently rising. Most patients present with localized disease which has a high survival after definitive treatment with chemoradiation.

For patients who develop metastatic disease or present with this de novo, survival is poor.

Areas covered: This review provides a summary of current literature on anal cancer. With a focus on women, this includes current epidemiological trends, role of HPV, and the current and future treatment landscape, including HPV vaccination and immunotherapy. Screening currently focusses on HIV-positive men, missing most female cases. In curative disease, trials are investigating treatment de-intensification in good prognostic groups. Immunotherapy is showing early promise in the advanced disease setting.

Expert opinion: Similar to cervical cancer, anal cancer is strongly associated with HPV and therefore, broader implementation of screening programs may reduce its incidence. HPV vaccination is expected to reduce the development of (pre)malignant anal lesions. The emergence of biomarkers will assist patient treatment selection, allowing optimal balance of treatment efficacy and morbidity. It is hoped that new treatment approaches, including immunotherapy, will improve outcomes. International collaboration is needed.

Article Highlights

- Anal cancer incidence has been rising for 40 years. It is more common in women than men, apart from selected high-risk populations such as HIV co-infection

- Squamous cell carcinoma is the predominant histology and strongly associated with HPV infection

- Currently screening is not targeted towards women despite their significant disease burden

- Chemoradiation remains the standard treatment in early disease, with surgical intervention reserved as salvage therapy

- Morbidity for localized treatment has reduced with improved radiation techniques but significant long-term quality of life impact remains

- In the metastatic setting survival remains poor. Newer treatment options such as immunotherapy are being evaluated with promising early results.

1. Introduction

Anal cancer is uncommon, but incidence has been increasing over the last four decades. Anal cancer comprised 0.3% of incident cancer diagnoses in 2018, totalling a global cancer burden of 48 541 new cases, 28 345 of them in women, and accounting for 0.2% of cancer□related deaths [1]. The majority of anal cancers (70-80%) are squamous cell carcinomas (SCC). Approximately 20% are adenocarcinomas, where the true origin may be the rectum. Primary anal adenocarcinomas may be considered in two distinct groups, those originating from the anal glands/transitional zone, and those with colorectal origin, the former group may be associated with HPV and treated similarly to anal SCC[2]. Those of colorectal origin should be treated as rectal cancer. The remainder are rare subtypes including melanoma and neuroendocrine tumours [3]. This review will focus on SCC as the most significant malignant disease in the anal region.

The Human Papilloma Virus (HPV) underpins the aetiology in most cases and vaccination of both boys and girls against HPV is predicted to dramatically decrease

future incidence. Human immunodeficiency virus (HIV) coinfection markedly increases the risk of HPV-associated anal SCC, however presentations associated with coinfection by HIV also peaked prior to control of HIV with antiviral therapy [4].

Most anal cancers present as localized disease and are treated with curative intent, with a high survival rate. Treatment has changed significantly, with organ preservation and reduction in toxicity being the major advances in recent decades. For patients with metastatic anal cancer, advances in systemic therapy and particularly the role of immunotherapy is of note.

This article summarises the pathophysiology, epidemiology, and treatment of anal SCC with a particular focus on the disease in women (Figure 1).

2. Pathophysiology

2.1 Anatomy

The anal canal extends from the distal end of the rectum to the external anal opening. Histologically, the proximal end of the anal canal contains both columnar and squamous epithelium forming the "transitional zone," which extends until the visible dentate line. The remainder of the anal canal is squamous epithelium, with a few anal glands which secrete mucous. It then transitions to the perianal skin which is hair bearing and contains apocrine sweat glands [5]. Anal SCC is considered to be cancer arising in the anal canal, and there appear to be two sites of origin: anal transitional zone and squamous mucosa of the anal canal [6]. Whilst basal cells have been thought to be the target cells of HPV in HPV associated pre-malignant and malignant lesions, there is evidence to suggest that specific and unique cells of the squamocolumnar junction are particularly susceptible to, and the target for, carcinogenic HPV and carcinogenesis [7,8].

2.2 Natural history

Progression from premalignant to malignant disease is acknowledged, although this is less well described than in cervical cancer. There are well described in situ lesions (anal intraepithelial neoplasia- AIN). These lesions can be divided into low risk (AIN 1, also known as low grade squamous intraepithelial lesion (LSIL) and high risk/ premalignant (AIN 2 and 3, also known as high grade squamous intraepithelial lesions (HSIL) [9]. AIN 1/LSIL is not associated with progression to invasive malignancy, whereas the HSILs are.

If excised, AIN has been found to have a low rate of recurrence and progression to invasive malignancy in immunocompetent hosts [10]. In a study of HIV-infected and uninfected men, with a median follow up of 1.1 years, progression of low grade AIN to high grade AIN was 7.4/100 person years, while regression of high-grade AIN was 23.5/100 person-years. 2/101 (1.9%) of high-grade AIN patients progressed to invasive SCC. Progression was more likely in HIV-infected patients, particularly with low CD4 counts [11]. Higher rates were reported in another cohort composed of 18 men and 52 women, with 11% progression to anal SCC and 25% regression. Progression was also associated with immunocompromise. The median time to progression was 42 months from diagnosis [12]. A large registry study (without details such as HIV infection) found that 9.5% of patients with AIN 3 could be expected to progress to invasive anal SCC within 5 years. This study found that prior ablative therapy reduced the risk of progression to cancer [13]. A more recent study, with median follow up of only 19.1 months, found that 2.9% of the cohort with HSIL progressed to cancer, however the median time to progression was 28.8 months [14] so this is likely to increase with longer follow up.

The true rate of progression is therefore likely to be around 10% over 5 years. There is a known latency period of approximately 15 years for CIN to develop into cervical cancer [15], and this could suggest that there is also a (perhaps longer than 5 years) latent period between development of AIN into anal SCC.

3. Epidemiology

3.1 Incidence and Mortality

The incidence of anal cancer in women has increased during the past 30 years and is more pronounced in high income countries. Worldwide, the incidence is 1.75 per 100 000 in females in high income countries, compared to rates less of less than 0.5 in Asia and central or eastern Europe, across both sexes [16,17]. The current incidence in the United States, per 100 000 is 1.5 in males and 2.1 in females, increasing by 2.7% per year [18,19]. The rise is mainly due to SCC, with adenocarcinoma incidence stable [17]. A recent US study reports that women comprise 65% of cases, with higher rates of anal SCC than males aged over 50 [19]. In the non-HIV infected population, women have higher rates of anal cancer than men in age groups over 30.

The mortality rate from anal cancer has also increased by 3.1% per year, with higher rates over 50 years of age, highlighting elderly females as a high risk group [19].

3.2 HPV

HPV is a small DNA virus that is able to integrate into a host genome. In particular, the E6 and E7 genes produced by high risk HPV persist, allowing "immortalization" of keratinocytes, first demonstrated in the 1980s in association with HPV 16 [20,21]. This confers genomic instability in the host, leading to accumulation of mutations [22]. The E6 and E7 proteins also play a role in allowing infected cells to evade the immune

response through various mechanisms, including interference with expression of toll-like receptors and interferon receptors [23]. A third oncoprotein, E5, also appears to play a role in assisting HPV virus immune evasion, including via a role in MHC/HLA class 1 downregulation and interference with cytokine production[24].

Molecular profiling by exome sequencing in 24 patients with metastatic SCC revealed *PIK3CA* as the most commonly mutated gene in HPV-positive tumors [25]. Also, mutations in genes important in histone modification such as MLL2 and MLL3 are frequently mutated. They are activated "early" in the HPV life cycle and promote immune evasion in infected anal epithelial cells [26].

HPV is strongly implicated in the development of anal cancer with the spectrum of HPV types similar to the cervix; HPV 16 is particularly carcinogenic. The prevalence ratio for anal cancers versus normal cytology was found to be 5 in a meta-analysis of 18646 patients with published HPV testing and anal cytology or histology results [27]. HPV16 was found in 86% of anal cancers in the HIV negative population and 67% in the HIV positive population. The next most common HPV subtype was HPV 18, present in 4% of HIV negative and 15% HIV positive patients. HPV 6 and 11 were present in 3-8% of anal cancers (less common in HIV negative population). One or more of the subtypes covered by the quadrivalent HPV vaccine (subtypes 6, 11, 16, 18) were found in 92% of anal cancers, and one or more covered by the nonavalent vaccine (subtypes 6, 11, 16, 18, 31, 33, 45, 52, 58) were found in 98% of anal cancer cases. Of patients with a single HPV strain only, 95% of cases in HIV negative patients would be covered by the nonavalent vaccine but only 75% in HIV positive patients [27]. It is speculated that the higher prevalence of HPV 16 in the HIV negative than in the HIV

positive anal cancer population is related to the ability of HPV 16 to evade normal immune functions [27].

Cross-sectional population data on anal HPV infection are lacking. The USA National Health and Nutrition Examination Survey uses data from cross-sectional population sampling, with data for genital prevalence of HPV based on penile or vaginal swabs. 2013-2014 data shows a prevalence of genital HPV infection slightly higher in men than in women (45.2% vs 39.9%), for high risk subtypes the prevalence was 25.1% vs 20.4% [28]. Prevalence alone therefore does not seem to explain the increased rate of HPV-associated anal cancer in women.

3.2.1 Cervical and anal HPV cross-infection

The increasing incidence in anal SCC over the past 50 years correlates with rising cervical HPV infection rates. The link between concurrent cervical and anal infection is recognized. Hypotheses include anatomical proximity and the high rate of transmission by hand, oral or penetrative sexual intercourse with cervical HPV acting as a viral reservoir for re-inoculation [29]. A recent systematic review of HIV-negative women in the general population found that anal HPV16 was present in 41% of women with cervical HPV16, compared with only 2% of cervical HPV16 negative women [30].

3.2.2 Reduced clearance of HPV in women

In healthy Hawaiian women, the median duration to clear anal HPV was 9.2 months and 74% of HPV infections were cleared within 1 year [31]. Clearance rates in HIV-negative men from Brazil and the USA at 6-months were 55.8% for men who have sex

with men (MSM) and 77.5% for men who have sex with women [32]. It is difficult to make cross study comparisons due to the different time intervals used and populations under study, but the lower rate of clearance in MSM suggests that reinfection through anal intercourse may be a factor in persistent infection.

A large cohort study of roughly 5000 men and women in China found that incidence of high-risk anal or perianal HPV infection was higher for women than men and clearance was lower, with a clearance rate ratio of 1.54 [33]. Interestingly all men with incident infection cleared it within a year, whereas 44% of women with incident infection were still infected one year later.

In the HIV-positive population, persistence of anal HPV was higher in women compared with heterosexual men (hazard ratio of 0.71, 95% CI 0.54-0.91), MSM and women had similar rates of persistent infection [34].

3.2.3 HPV vaccination

Landmark trials published in 2007 and 2009 of the quadrivalent and bivalent HPV vaccines proved efficacious in prevention of cervical HSIL in HIV positive women, with much greater efficacy of 98% in the per-protocol (PP) population of women who did not yet have HPV 16/18 infection of cervical dysplasia, compared to the overall population at 42% [35]. There is no specific data on HPV vaccination preventing anal cancer in women, but a large randomized controlled trial in men has shown efficacy in the PP population of 77.5% in preventing anal HSIL, and 94.9% in preventing persistent HPV infection associated with the targeted HPV subtypes 6, 11, 16 and 18 [36].

A potential role for vaccination as secondary prevention has also been demonstrated. A cohort study looked at quadrivalent HPV vaccine and recurrence of previously treated high-grade anal epithelial neoplasia in a MSM population, showing that vaccination lead to reduced risk of recurrence (HR 0.50; 95% CI, 0.26-0.98;

p=0.04) [37]. In patients with oncogenic HPV infection, vaccination was associated with a trend towards reduced risk of disease recurrence (HR 0.47; 95% CI 0.22-1.00; p=0.05)[37]. In a systematic review of HPV vaccination for secondary prevention, in most but not all, of the included studies there appeared to be a benefit in the adjuvant setting [38]. Further prospective studies are warranted.

HPV vaccination is most effective when given prior to HPV infection and is recommended before sexual debut. Retrospective analysis of this trial found that vaccination also reduced the risk of subsequent HPV-related genital lesions by 46% in women who underwent surgery for HPV-related genital lesions via prevention of re-infection, although anal HSIL or cancer was not included as a data endpoint [39]. In the older population, vaccination later in life does not seem to prevent reactivation of latent infections [40].

A possible adjuvant role of the quadrivalent HPV vaccine is demonstrated by a prospective case-control study ("SPERANZA"). Quadrivalent HPV vaccination administered to women after surgical management with cervical conization (LEEP: loop electrosurgical excision procedure) for grade 2 or greater cervical intraepithelial neoplasia in a case-control study (where the intervention group chose to have the vaccination; the control group declined vaccination)[41]. Vaccination resulted in a significant reduction in risk of subsequent HPV related high-grade CIN by 81.2% (95% CI 34.2-95.6), with rates of disease recurrence in the vaccination group of 1.2%, and in the non-vaccinated group 6.4% [41]. A small, nonrandomised cohort study of 202 patients with high-grade AIN showed fewer subsequent diagnoses at 2 years (HR 0.50; 95% CI, 0.26–0.98; p = 0.05) following quadrivalent HPV vaccination [42]. A separate study of 737 patients with CIN2/3, treated with LEEP, also showed reduced rates of

subsequent lesions in patients who subsequently received the quadrivalent HPV vaccine compared with a nonvaccinated group [43].

HPV vaccination is now recommended for all girls in the USA, however in 2010 only 48.7% of girls aged 13-17 received 1 or more doses of the HPV vaccine, and over the 2000-2009 period HPV-related oropharyngeal, vulval and anal cancer rates increased [44]. Higher vaccination uptake, the inclusion of boys in immunization programs to increase herd immunity, and more latency time may be required to assess whether HPV vaccination will successfully reduce the incidence of anal cancer. In the area of cervical cancer, rates over the past 10 years are decreasing but confounders include increased uptake in Pap screening and treatment of cervical HSIL [44].

In Australian women, the population-wide implementation of HPV vaccine has already resulted in significantly lower rates of HPV infection in young women in Australia. The prevalence for women aged 18-24 declined from 22.7% in 2005 to 1.5% in 2015 [45], which has been accompanied by declining rates of cervical dysplasia [46]. It is anticipated that similar reductions in rates of anal dysplasia will be observed in the future.

3.2.4 HIV co-infection

HIV infection is a strong risk factor for anal HPV infection and anal SCC. A meta-analysis of studies encompassing 18 646 men and women with anal cancer across 5 continents found that anal HPV was more prevalent in HIV-positive women than HIV-negative (100% vs 90%), and that HIV-positive individuals were also more likely to be infected with multiple HPV subtypes (42% vs 10%). The high-risk HPV16 was found as the single subtype in 42% of HIV-positive subjects compared to 78% of HIV-

negative, which may be explained by the ability of HPV16 in particular to evade host immune control [27].

Although HIV-infected women are a high-risk population with a relative risk of 24 for anal cancer compared to healthy women, the absolute numbers of HIV-infected women are lower than men (0.2% vs 0.5% of the population in North America) [47]. HIV co-infection therefore does not account for the overall rise in anal SCC incidence in women. A cohort study in the USA covering the period 1980 to 2005 found that incidence increased in women by 3.3% per year, regardless of HIV infection rates. In the latter 5 years only 1.2% of women with anal cancer were also HIV-positive, compared with 28% of men [47].

3.3 Sexual behaviors and sexually transmitted infections

Changes in sexual behavior with a lower age of first sexual intercourse, and increased number of sexual partners since 1950 have coincided with an increase in the rates of anal cancer as well as vulval cancer [16]. Anal intercourse is a risk factor for anal SCC in women without concurrent cervical HPV infection [29]. Another suggested explanation for higher rates of anal SCC in women than men is a higher rate of receptive anal intercourse in the age cohort over 50 years (35% vs 10%) [47].

A case-control study in Denmark and Sweden identified sexually transmitted infections as risk factors for anal SCC in women. Risk factors included greater than nine lifetime sexual partners; sexual partners with a history of sexually transmitted infection (STI); and a personal history of other STI including anal warts, genital warts, gonorrhea, chlamydia and cervical dysplasia [48].

3.4 Gynecological malignancy and the role of estrogens

A retrospective database analysis on women in high income countries with cervical cancer had an increased risk ratio of 3.12 for subsequent anal cancer [49]. In the Asian population with a lower prevalence of HPV-related cancers, a Taiwanese study reported a risk ratio of 1.36 for anal cancer after prior cervical cancer[50].

In keeping with a common developmental pathway of anal and cervical cancers, the development of anal HSIL was found in 22% of women with cervical HSIL compared with 1% of those without [30]. In a Dutch cohort study, women with a history of cervical HSIL grade 3 followed over 20 years have a subsequent risk ratio of 3.85 for anal cancer and 6.68 for anal HSIL [51]. A prospective analysis using data form the Million Women Study found a relative risk of 4 for anal cancer in women with premalignant grade 3 cervical HSIL [52]. This study also reported relative risks of around 1.5 for anal cancer with a history of oral contraceptive use, nulliparity, tubal ligation, and living alone [52].

The role of estrogens in anal cancer is not well established. One study reported that exogenous oral contraceptive use was associated with relative risk for anal cancer of 1.5, which increased to 1.68 with use longer than 5 years [52]. However, another Danish study notes that anal SCC risk was higher in women with late menarche or low body mass, suggesting endogenous estrogen may have a protective effect, and more research is needed in this area [53].

3.5 Smoking

Smoking has been implicated in both reduced clearance of anal HPV [32], and directly as a risk factor for anal cancer with a relative risk of 1.5 [52,54]. There may be an interaction between smoking and estrogen in younger women, as smoking was shown to increase the risk of anal SCC in premenopausal women but not postmenopausal women

[53]. Smoking may also have a prognostic effect, and a small retrospective review of anal SCC patients noted that smokers had slightly higher rates of cancer recurrence and were more likely to die from disease [55].

3.6 Immunosuppression

A meta-analysis of cancers of immunosuppressed transplant recipients versus HIV positive individuals was conducted aiming to distinguish cancer risk based on HIV infection alone compared to the associated immunosuppressed state. HPV-associated cancers, including cervical, anal and oropharyngeal SCC were observed at similar rates, suggesting that immunosuppression rather than HIV infection per se accounts for the increased risk of viral oncogenic cancers [56].

3.7 Anal cancer as a risk factor for subsequent malignancy

Patients are at risk of developing a further malignancy associated with HPV. American registry data over 1973-2000 found that women with anal cancer had observed/expected (O/E) ratios of 1.3 for subsequent gynecological cancers and 1.7 for nasopharyngeal cancers, both of which are also associated with HPV. There was also high O/E ratios for smoking-associated cancers; including gastro-esophageal (around 1.8) and lung cancer (2.3) [57].

In a systematic review and meta-analysis, patients diagnosed with a HPV related malignancy were at a five-fold risk of developing a second HPV related malignancy [43]. Notably, there is a strong association between anal and vulvo-vaginal cancers, where an initial diagnosis at one site confers around a ten-fold risk of a second malignancy at the other site [43]. Implications following an initial diagnosis of a HPV

malignancy therefore, include screening strategies and secondary prevention (including utilization of vaccines).

Reasons for increased risk of second HPV related malignancy may be related to exposure to known risk factors including sexual practices, but also potentially related to patient genetic susceptibility. MHC haplotypes can be protective or associated with higher risk [58], and a variant of the CXCL12 gene conferring higher risk also [59]. Such factors could be utilized when trying to identify patients at high risk of disease.

4. Screening

There are several tests that have the potential to be used as screening for anal SCC and precursor lesions.

4.1 Digital examination

Digital examination is cheap and easily accessible regardless of healthcare setting. A study in Australian MSM reported that this test was generally acceptable to patients, with minimal discomfort (similar data in women is lacking) [60]. The Study of Prevention of Anal Cancer prospective cohort study of anal cancer screening in Australian MSM will add systematic data regarding the sensitivity and specificity of digital examination in comparison to cytology and anoscopy [61].

4.2 Anal cytology

Anal cytology can be collected using a blind swab of the anal canal and suspension of cells in liquid medium. This technology is similar to cervical screening. A meta-analysis of 30 studies incorporating data from 4074 patients found the overall sensitivity of the

test for HSIL vs normal anal epithelium to be high (85%) but specificity to be low (43%). Sensitivity was slightly higher in the HIV-positive population (88.2%) [62]. A study in high-risk women comparing high-resolution anoscopy to anal cytology found that sensitivity and specificity of cytology were similar to that found in the meta-analysis. Adding testing for high risk HPV to cytology increased sensitivity to 100%, but reduced specificity to 16.8% [63], although this has not been a consistent finding [64].

There is poor correlation between grade of abnormality on cytology versus biopsy. A study of 278 women at high risk of anal cancer due to prior anogenital neoplasia found that "any abnormality" on cytology had 71% sensitivity and 73% specificity for HSIL or cancer on histology. However, sensitivity of "any abnormality" on cytology to detect "any abnormality" on histology was only 47%. Sensitivity was higher in those with concurrent genital HSIL or cancer (88%). The kappa statistic for concordance between grades on cytology versus histology was 0.147 (poor). Forty-four percent of women with low grade abnormalities on cytology were found to have high grade abnormalities on histology [65].

The sensitivity and specificity of anal cytology is affected by the pre-test probability of anal cytologic abnormalities [62,65]. In the general population, the pre-test probability of abnormalities is only 4% [66]. Various risk factors for anal cancer in the studies above (HIV, concomitant genital HSIL) increased the sensitivity of cytology. Combining this with clinical assessment of risk factors may form the basis of screening that can be added with limited additional expertise and facilities beyond those required for the widely implemented cervical screening programmes.

4.3 High resolution anoscopy

High resolution anoscopy is a technical procedure using similar techniques to cervical colposcopy, with some additional technical challenges related to the anatomy of the anal canal [67]. It is performed using a modified gynaecologic colposcope and an anoscope (analogous to the vaginal speculum). Acetic acid is used to highlight areas of abnormal epithelium, which can be further delineated with Lugol's stain [68]. The technique has a significant learning curve as well as being time consuming and therefore is restricted to examining the anus after the abnormalities are found on cytology, or in a research setting [69].

4.4 Management of HSIL found during screening

The goal of screening is to identify patients with precancerous lesions or very early-stage cancer. The management of premalignant lesions found during screening is controversial, due to a lack of prospective randomized data and the question remains of spontaneous regression from high grade HSIL to lower grade abnormalities. The ANCHOR study of 5058 HIV positive patients with HSIL has randomized participants to active surveillance or local therapy with topical treatments or ablation [70]. The aim of this study is to assess active surveillance is adequate for these patients versus early intervention, with an endpoint of time to anal cancer.

There are no large completed studies of intervention in anal HSIL. A randomized study of infrared coagulation showed that it is more effective at clearing HSIL than observation alone [71], however this study was not designed to show a difference in risk of progression to invasive cancer. A number of topical therapies are in clinical use including topical 5-FU, imiquimod, cidofovir and trichloroacetic acid. The use of these agents is largely extrapolated from their use in other mucocutaneous sites and the data in anal cancer is limited to case series and single arm studies [72].

A different approach to secondary prevention is use of the HPV vaccine in patients with HSIL. A nonrandomized cohort study of HIV positive MSM with a diagnosis of high grade AIN (which had been treated with local excision or targeted ablation) showed the rate of recurrence of high grade AIN was 13%, compared with 30% in unvaccinated men [42].

There is also potential for therapeutic vaccines in the HSIL setting, which aim to induce cell-mediated immunity against pre-malignant cells. There is ongoing research into an appropriate target (the E6 and E7 proteins have been suggested as potential choices) and the induction of an adequate immune response[73]. As yet, no therapeutic vaccines are available for clinical use but are under investigation.

5. Diagnosis

Most patients have localized disease at presentation. Common presenting symptoms of anal cancer include bleeding, lump (often assumed by patients to be hemorrhoid) and anorectal pain. Many patients are asymptomatic with the diagnosis made incidentally when patients are examined for other reasons. Physical examination should include Pap smear and colposcopy in women, to exclude a concurrent cervical lesion. Biopsy is required for histological diagnosis. It is not uncommon for the diagnosis to have been made of rectal cancer with a biopsy then showing SCC and careful review of location revealing an anal primary site.

A diagnosis of anal cancer is accompanied by stigma and a sense of shame amongst many patients [74,75]. Likely contributory factors are the anatomical location, rarity and subsequent poor disease understanding as well as link with HPV and sexual activity. This may impact the patient experience of treatment and survivorship, an area requiring further evaluation [76].

6. Staging

Anal cancer is staged according to the tumor, node, metastasis (TNM) system devised by the American Joint Committee on Cancer (AJCC) and the Union for International Cancer Control (UICC). Tumor size (T) and nodal status (N) are important prognostic factors [62,63]. The latest eighth (8th) edition of the AJCC staging subclassified stage II disease into IIA (T2N0M0, T2 defined as primary size > 2 and ≤5cm) and IIB (T3N0M0, T3 defined as primary size > 5cm) due to their prognostic differences[77] . A National Cancer Database (NCDB) and Surveillance, Epidemiology, and End Results (SEER) study demonstrated 5-year overall survival (OS) rates for stage IIA of 72% and 69% and IIB 57% and 50% in the NCDB and SEER databases respectively [78].Patients with stage IIA disease were more often female as compared with stage IIB patients [78].

Multiple imaging modalities are utilized for clinical staging and treatment planning, and currently magnetic resonance imaging (MRI) and computed tomography (CT) are standard. MRI of the pelvis is important to assess locoregional disease, including tumor size, infiltration, and sphincter and nodal involvement [79].

More recently positron emission tomography (PET) or integrated PET/CT is being utilized. A meta-analysis of 12 studies reported that PET or integrated PET/CT lead to upstaging or downstaging of nodal disease in 28% of patients (95% CI 18-38%) [81]. This has implications for treatment planning, notably for inclusion of involved lymph nodes in the radiation field. Furthermore, PET imaging demonstrating large metabolic tumor volume is associated with a poorer prognosis [82,83].

7. Treatment
7.1. Localized disease

Following a landmark trial showing equivalence of organ preservation with surgical resection (abdominoperineal resection necessitating permanent stoma), the standard of care in localized and locally advanced (LA) anal SCC for many years has consisted of curative intent (definitive) concurrent chemoradiation [84]. The chemotherapy consists of infusional 5-fluorouracil (5FU) and mitomycin C (MMC) given during weeks 1 and 5 of radiation [85-89]. More recently, the substitution of capecitabine for 5FU has provided an alternative that frees the patient from requiring central venous access [90-94]. Trials of substituting agents such as cisplatin surprisingly reported inferior results [86,89]. In addition, no benefit was seen to neoadjuvant chemotherapy prior to chemoradiation, nor to adjuvant chemotherapy cycles beyond the definitive treatment [86,95,96].

7.1.1 Advances in radiation

Definitive chemoradiation is associated with significant acute, medium and long term toxicity. The original radiotherapy treatment protocols, as described by Nigro et al, resulted in significant toxicity with a high incidence of hospitalization [84,97]. This includes haematological toxicity resulting from radiation exposure to bone marrow with subsequent myelosuppression; acute skin reactions with subsequent pain and requirements for skin dressings; genitourinary toxicity which includes acute bland cystitis; and gastrointestinal toxicity, commonly diarrhoea. In the RTOG-9811 trial (utilizing conventional radiation) in patients receiving chemoradiation with concurrent MMC/5FU, rates of acute grade 3 and 4 haematological and non-haematological toxicity were 61% and 74% respectively [86].

Subsequently, with conventional radiation techniques, treatment intensity was often impacted, requiring breaks or early cessation, which translated into inferior outcomes including poorer locoregional control [98-100]. A recent cohort study of 1125

patients undergoing definitive chemoradiation between 2007 and 2015 for anal SCC confirms a detrimental effect [101]. They found that 18% did not complete radiation and 25% did not complete chemoradiation [101]. Patients less likely to complete chemoradiation were those aged over 70 years old (risk ratio [RR] 0.6; 95% CI 0.52-0.70) and those with more comorbidities (RR 0.70; 95% CI 0.51-0.95)[101]. Incompletion of chemoradiation was associated with significantly higher risk of need for salvage surgery (RR 1.54; 95% CI 1.03-2.31), cancer specific death (HR 1.59; 95% CI 1.14-2.22), and colostomy or death (HR 1.80; 95% CI 1.10-2.93)[101].

Intensity modulated radiotherapy (IMRT) has allowed the much more targeted delivery of radiation with sparing of nearby organs and reduced toxicity [102,103]. Its efficacy with concurrent chemotherapy was demonstrated in the RTOG-0529 study, with a four year OS rate of 85.8% and four year disease free survival of 75.5%[104]. Just over two thirds of these patients were female; on univariate analysis, sex was not significantly associated with inferior OS or event free survival. Compared with the RTOG-9811 trial utilizing conventional radiation, this trial reported a significant reduction in grade 3 or more GI toxicity (21% vs 36%; p=0.0082); grade 3 or more dermatologic toxicity (23% vs 49%; p<0.0001); and acute grade 2 or more haematologic toxicity (73% vs 85%, p=0.032) [104]. Treatment breaks for acute toxicity were required in 49% of patients in RTOG-0529 compared with 62% in RTOG-9811, which was not statistically significant. The duration of treatment breaks however was significantly shorter in the RTOG-0529 group [104].

A cohort study of 99 patients utilizing IMRT as per the RTOG-0529 study provided further support for this approach, with grade 3 and 4 acute haematologic and non-haematologic toxicity rates of 63% and 21% respectively [103]. Grade 1 diarrhoea was the most common late toxicity which was reported in 37% of patients. Almost 94%

of patients had a complete clinical response to treatment, and the 4 year event free survival and OS rates were 75.5% and 85.8% respectively [103]. IMRT is now adopted as the standard.

With high cure rates of definitive chemoradiation, chronic toxicities affecting quality of life are common and significant problem. Some patients experience these late effects for years after completion of treatment[105]. Bowel dysfunction (including chronic diarrhea, fecal incontinence and urgency) as well as urinary frequency and incontinence can cause significant distress in survivors [106-108]. Sexual dysfunction is reported to affect half of female patients[107-109]. Owing to the close proximity of the vagina to the anal canal, toxic mucosal effects of radiation can result in vaginal stenosis and dyspareunia[110]. Loss of libido is another consequence, likely a combination of physical toxicities and psychosocial impacts of treatment [111]. Thirty to forty percent of sexually active women become sexually inactive following completion of chemoradiation for anal cancer [112]. Sexual dysfunction is also an issue amongst male survivors, with erectile dysfunction being common [106].

7.1.2 Assessment of treatment response to chemoradiation

The best timing of post treatment response to therapy is approximately 26 weeks from starting chemoradiation, with data demonstrating ongoing tumour response to the 6 month mark [113,114]. The best modality for tumour response assessment is currently with MRI [115]. A retrospective study demonstrated that MRI repeated 3 and 6 months post definitive chemoradiation, with application of tumor regression grading scores, predicted early local tumor relapse [80]. A role for PET in assessment of treatment response has not been strongly established. A small prospective trial of 19 patients with PET imaging prior to and then 12 weeks following chemoradiation demonstrated that PET parameters predicted local recurrence [116].

Pre-treatment imaging findings may also be useful in predicting outcomes following chemoradiation. A retrospective study has demonstrated that tumour texture features assessed by MRI were prognostic [117]. A retrospective study of patients who received FDG PET/CT staging prior to curative intent chemoradiation for anal SCC demonstrated that extracted radiomic features were predictive of PFS [118]. Further studies may help define role for PET imaging in the assessment in anal cancer.

7.1.3 Salvage therapy for local relapse

For patients who experience locally recurrent or persistent disease following definitive chemoradiation, surgery with abdominoperineal resection (APR) is recommended [119]. In this clinical context, single institution retrospective studies have demonstrated 5-year survival rates of 29-64% [119-122] . No significant difference in outcomes has been demonstrated for females compared with males [120,122]. It is important to wait adequate periods before repeat biopsy for residual disease, with the ACT II trial showing that the six month rate of residual disease was 22% compared to 48% at 3 months [113].

7.2 Biomarkers

Identifying biomarkers for subgroups of patients who may respond better or less to treatments is the subject of active investigation.

HPV positivity and p16 expression (which is frequently used as a surrogate for HPV positivity) is correlated with improved OS and PFS/DFS in anal SCC [124,125]. In a recent meta-analysis, HPV positivity was associated with superior locoregional recurrence rates compared with HPV negative tumours (HR 0.27; 95% CI 0.16-0.48, p<0.001), OS (HR 0.26; 95% CI 0.12-0.59, p=0.001) and DFS (HR 0.33; 95% CI 0.16-

0.70)[126]. When assessing p16 status, outcomes were superior for p16 positivity, with reduced locoregional recurrence (HR 0.26; 95% CI0.13-0.52; p<0.001), superior OS (HR 0.44; 95% CI 0.24-0.81; p=0.009) and DFS (HR 0.44' 95% CI 0.23-0.83; p=0.012)[126].

Anal SCC which is HPV and p16 negative have worse outcomes following definitive chemoradiation, and they more commonly harbor TP53 mutations, another negative prognostic factor [124,126-128]. A more optimal treatment approach is needed for these patients, where there is clearly a different biology.

Circulating tumour DNA (ctDNA) is an attractive biomarker due to being non-invasive and assessible at multiple time points. Circulating HPV DNA (HPV ctDNA) was prospectively assessed as a biomarker in patients receiving first line chemotherapy with docetaxel, cisplatin and 5-fluorouracil (DCF) in metastatic or recurrent locally advanced anal SCC [129]. In this study pre-treatment HPV ctDNA levels were higher in patients with metastatic compared to recurrent locally advanced disease, but did not predict for treatment response radiologically [129]. Of interest, HPV ctDNA was predictive for sustained treatment response following completion of chemotherapy. Median PFS was superior in patients with undetectable compared with detectable levels of HPV ctDNA (PFS HR 5.5; 95% CI 2.1-14.3, p<0.001) [129]. One year OS rates in patients with undetectable and detectable HPV ctDNA were 87% and 50% respectively [129]. Baseline HPV ctDNA was not prognostic.

Immune biomarkers have also been demonstrated to be associated with prognosis in patients undergoing definitive chemoradiation for anal cancer. This includes the neutrophil:lymphocyte ratio, where a higher pre-treatment ratio has been associated with poorer outcomes [130-132]. Likewise, tumour infiltrating lymphocyte scores appear prognostic. A study of immune biomarkers in 150 patients with HPV

positive anal SCC reported strong association between high CD8+ and PD-1+ tumor infiltrating lymphocytes (TIL) expression and improved local disease control and disease-free survival after definitive chemoradiation [123]. TIL score therefore may be clinically relevant in helping to risk stratify p16 positive patients, and assist in patient selection for dose de-escalation in favourable risk patients [123,133].

7.3 Metastatic disease and unresectable local disease

For patients with metastatic disease or inoperable recurrent local disease, treatment intent is palliative and involves systemic therapy with median survival rates of approximately 12 months [134-136]. Metastatic anal cancer, either de novo or following previous definitive treatment is a rare cancer, rendering the undertaking of clinical trials difficult. Additionally, patients with HIV infection, who constitute a significant population with anal cancer, have traditionally been excluded from trials.

In an attempt to overcome these barriers and harness global participation to ensure adequate enrolment, the International Rare Cancers Initiative Metastatic Anal Cancer Working Party was established (https://www.cancer.gov/about-nci/organization/cgh/research/irci). The group undertook the first global academic trial in metastatic anal cancer, recruiting 91 patients (stratifying for HIV) from the United States, United Kingdom and Australia, to the InterAACT study, a randomized phase 2 trial comparing carboplatin plus paclitaxel to the standard cisplatin plus infusional 5FU in the first line metastatic cancer setting [137]. The response rates (primary endpoint) were similar between treatment arms, however overall survival (OS) was found to be superior in the carboplatin plus paclitaxel cohort (20 months vs 12.3 months, HR 2.0; p=0.014)[137]. This has been incorporated into the most recent National

Comprehensive Care Network guidelines (v1 2020), which note carboplatin and paclitaxel as the preferred regimen.

Alternative regimens, with limited evidence, include FOLCIS (employing lower dose cisplatin with 5FU) [138], modified FOLFOX (5FU and oxaliplatin) [139], and modified DCF (docetaxel, cisplatin and 5FU) [140]. These regimens are also used in second line treatment, depending on prior drug exposure.

7.4 Future directions

7.4.1 Immunotherapy

There is strong rationale for the utilization of immune-oncology (IO) agents, including the response of SCC from other anatomical sites. Biological rationale includes oncogenic HPV proteins (notably E6 and E7) being able to stimulate T cell reactivity and upregulate inhibitory checkpoint molecules with subsequent reduction in cytotoxic T cell responses [141,142]. Furthermore, the immune responses are also known to play a role in preventing chronic HPV infection progressing to invasive malignancy [143]. The use of IO in patients with HIV was initially concerning, with reports of increased HIV transcription (potentially through HIV latency reversal), however this appears to be transient and there are promising results with longer follow up of IO agents in patients with HIV and malignancy in terms of efficacy and safety [144].

7.4.2 Checkpoint Inhibitors

This class includes programmed death ligand-1 (PD-1) inhibitors (such as nivolumab and pembrolizumab); programmed death ligand 1 (PD-L1) inhibitors (such as. atezolizumab and durvalumab) and cytotoxic T lymphocyte associated protein 4 (CTLA4) inhibitors (such as ipilimumab). These agents are now standard of care for

many cancers, having dramatically improved outcomes, including squamous cell skin cancers, lung cancer and melanoma.

Twenty-five patients (23 female) with previously treated advanced anal SCC were enrolled in the KEYNOTE-028 phase 1b trial investigating pembrolizumab in tumours with PD-L1 expression of ≥1%. Anti-tumour activity was demonstrated with overall response rate (ORR) of 17% (95% CI, 5-37%) and a further 42% of patients experiencing stable disease [145]. Median progression free survival (PFS) at 6 and 12 months were 31.6% and 19.7% respectively [145]. The cohort of 112 patients with anal cancer in of the KEYNOTE-158 phase 2 trial of single agent pembrolizumab, reported at median 12 months follow up, showed an ORR of 11.6% (95% CI, 6.3-19%), median OS 12 months (95% CI, 9.1-15.4 months) and median PFS of 2 months (95%CI, 2-2.1 months) [146]. Patients with HIV were excluded from both studies.

Single agent nivolumab was investigated in a phase 2 study of 37 patients with previously treated, advanced anal cancer SCC. Patients with stable HIV were allowed on study, although only two were recruited. Criteria were: CD4 counts ≥300/uL, undetectable viral load, receiving antiretroviral therapy and under the care of an infectious disease specialist. This trial demonstrated an ORR of 24% (95% CI 15-33), with six month PFS of 38% (95% CI, 24-60). The median OS was 11.5 months (95% CI 7.1-not estimable), with estimated one year OS 48% [147]. These results are somewhat promising in this heavily pre-treated patient group.

There are at least five ongoing clinical trials raging from the refractory setting, to investigating IO in earlier metastatic settings; adding IO to chemotherapy and/or epidermal growth factor receptor inhibitors; and using combination IO agents (Table 1). With clinical activity of checkpoint inhibitors demonstrated in early trials in the advanced disease setting, it remains to be seen if these agents can improve outcomes in

patients with earlier stage disease, that is, reducing rates of disease recurrence and persistent disease post definitive chemoradiation. Studies are currently investigating checkpoint inhibitors in addition to chemoradiation in patients with high risk earlier stage disease, defined by larger primary tumours and/or nodal involvement (NCT04230759, NCT03233711).

7.4.3 Radiation Therapy

Whether treatment can be de-escalated in patients with early stage anal SCC is being studied in a phase 2 trial of patients with T1-2N0M0 anal SCC comparing standard chemoradiation (28 fractions of IMRT plus MMC administered day 1 plus either 5FU administered on days 1-4 and 29-32 or capecitabine 5 days per week during IMRT) with de-intensified chemoradiation consisting of 20 or 23 fractions of IMRT plus MMC on day 1 only and either 5FU days 1-4 or capecitabine 5 days per week during radiation (NCT04166318). Additionally, the PLATO umbrella trial is investigating radiation dose de-escalation in early disease (T1-2N0), and radiation dose escalation in more advanced disease (T3/4 N1-3)[148].

7.4.4 Cellular Therapies

This promising and relatively new immunotherapeutic approach is being investigated in HPV positive cancers, including anal SCC. Genetically engineered T cells are designed to specifically target tumor antigens (including E6 and E7 HPV oncoproteins) and include chimeric antigen receptor (CAR) or T cell receptor (TCR) T cells (for an excellent review, see Hinrichs et al)[149].

In a phase I/II study by Doran et al, 12 patients with advanced HPV positive cancers, including four with refractory anal cancer, were treated with a TCR T cell

targeting HPV oncoprotein E6. The treatment regimen included non-myeloablative chemotherapy as well as cytokine administration. Two patients had an OR (both with anal SCC), one of which was durable [150,151]. Interestingly, further evaluation of non-responders identified genetic defects in antigen presentation, T cell PD-1 expression and IFN-gamma response [151]. TCR T cells targeting HPV E7 are currently being investigated in an ongoing phase I/II clinical trial in HPV positive cancers including anal cancer (NCT02858310).

The ongoing HESTIA trial (NCT02379520) is investigating T cells engineered to recognize both HPV oncoproteins E6 and E7, with or without lymphodepletion and nivolumab, in patients with HPV associated cancers. Interestingly, T cells are also engineered to be resistant to TGF-b, and it remains to be seen if this approach will lead to meaningful clinical benefit with reduced treatment resistance.

Adoptive T cell therapy has been investigated in a phase 2 trial of patients with metastatic HPV positive cancers, with two cohorts of patients: cervical (n=18) and non-cervical cancers (n=11). Patients were required to have tumor surgically removed for subsequent tumor infiltrating lymphocyte (TIL) production. Patients received a single IV infusion of tumor TILs with reactivity directed at HPV oncoproteins E6 and E7, with a regimen that also included lymphodepleting chemotherapy and cytokine administration [152]. Of the 29 patients in this study, 7 had an OR. Although these are early and small studies, the demonstration of tumor activity is promising and results from further studies employing novel treatment combinations is anticipated.

7.4.5 Therapeutic Vaccines

Therapeutic vaccines are also a relatively new approach in HPV positive tumors including anal cancer. Generally, these involve exposure of HPV E6 and/or E7 to

antigen presenting cells with the aim of subsequent cytotoxic T cell activation and response. Pre-clinical studies have shown anti-tumor responses with such vaccines [153]. There is also interest in combining therapeutic vaccines with other immunotherapeutic agents. A pre-clinical study by Bartkowiak et al investigated an E6/7 HPV vaccine in combination with a checkpoint inhibitor or the tumor necrosis factor receptor family member 4-1BB agonist antibody. The latter combination demonstrated significant tumor regression including that was durable [154]. There are a number of ongoing trials utilizing therapeutic vaccines in HPV positive malignancies (NCT02865135, NCT03439085, NCT04180215).

8. Conclusion

Improved understanding of anal cancer, a disease that disproportionately affects women due to the central role of HPV infection, has allowed development of targeted prevention and treatment strategies. Implementation of HPV vaccination on a population level should decrease incidence of premalignant and malignant lesions, however this will take many years to translate into a reduction in cancer cases and death. In the meantime, the treatment landscape is improving for patients diagnosed with both early and late stage anal cancer. Treatment of early disease has high cure rate but still is associated with significant short and long term morbidity. A particular focus relates to the efficacy of immunotherapy in other squamous cancers, which is now being tested in various anal cancer settings. The psychosocial impact of the diagnosis and treatment sequalae of anal cancer is a significant issue, an enhanced understanding will help improve the patient experience.

Expert Opinion

The incidence of anal cancer in women has been increasing for several decades and is now higher than in men. Similar to cervical cancer, it is strongly associated with human papillomavirus (HPV). Therefore, like cervical cancer, anal cancer may be potentially preventable through screening of premalignant lesions. In the past most focus has been on men who have sex with men (MSM) and immunocompromised patients who are known to be at higher risk for anal cancer. Further research is needed to determine if screening of healthy women would reduce the risk of anal cancer and whether it is cost-effective. Although there are no official guidelines for anal cancer screening, we would advise screening in high risk female patients such as those who are immunosuppressed, have cancers of the lower genital tract and those with HPV positivity demonstrated on Pap smears. The optimal way of screening is not clear, neither is the optimal age to start screening. Therefore, further clinical research is urgently needed, and guidelines should be made.

Primary prevention by controlling the cause of anal cancer is undoubtably the best strategy for the future. Population wide HPV vaccination is known to have decreased rates of cervical premalignant lesions; the expectation is similar for anal intraepithelial neoplasia and hence progression to anal cancer. However, there are no data yet in females if the development of anal SIL could be decreased, but we anticipate it does. Screening and early intervention are the subject of large prospective trials and will provide robust data to inform targeted programmes.

Given the rarity of this disease both screening and treatment should be performed by centres with expertise in anal cancer. Experience is important in the

interpretation of anal cytology and the use high-resolution anoscopy, as indicated for biopsies, ablative treatments and posttreatment surveillance.

Treatment of localized disease has made significant advances, however the organ preservation and cure rate is still below 90%, particularly for some subgroups. Treatment☐related morbidity confers long term functional consequences; advances in radiation techniques should continue to reduce these. De☐intensification of treatment for very early disease is also likely to be shown to be effective whilst also reducing treatment adverse effects. Randomized trials addressing this are currently recruiting. Furthermore, we hope to see an improvement in long term outcomes for those patients with high risk disease, with current trials investigating the addition of checkpoint inhibitors to standard definitive chemoradiation regimens.

Patients with metastatic anal cancer should also be strongly advised to participate in clinical trials evaluating newer treatments such as checkpoint inhibitors. In the next five to ten years we anticipate such immunotherapeutic agents will become standard in the treatment of anal cancer. Clinical trials are currently investigating these agents in advanced anal cancer and are expected to change the treatment landscape, with the hope for durable responses and improved survival. Despite the limited experience in this disease, we foresee the results of such trials to be consistent with the experience of anti-PD1 in head and neck cancer which is another HPV related malignancy.

Patient recruitment to clinical trials is made difficult by the rarity of this disease. International collaboration in anal cancer is therefore paramount to ensure adequent patient recrtuiment. Such an approachhas been shown possible by the InterAACT study. . Biomarkers are still elusive in providing patient selection for precision treatment pathways but will emerge through further studies.

Finally, removing the stigma from anal cancer and HPV associated conditions is important, and health programs and education will be key. This will assist in making disease prevention and screening programs more accessible and acceptable, as well as ultimately improving patient outcomes and experiences.

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Declaration of Interests

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Tables/Figures

Table 1. Ongoing clinical trials investigating checkpoint inhibitors in advanced anal

SCC[155]

Clinical trial number	Phase	Patient cohort	Treatment arms	HIV eligible
NCT02919969	2	Metastatic, pre-treated Anal SCC	Pembrolizumab	Yes
NCT02314169	2	Metastatic, pre-treated Anal SCC	Nivolumab vs Nivolumab plus ipilimumab	Yes
NCT03944252	2	Unresectable, locally advanced or metastatic anal SCC, second line treatment setting	Cetuximab plus avelumab vs Avelumab	Yes
NCT03427411	2	Locally advanced or metastatic HPV associated malignancies	M7824 (novel bifunctional anti-PD-L1/TGFβ Trap fusion	Yes

		including anal SCC	protein)	
NCT03519295	2	Unresectable locally advanced recurrent or metastatic anal SCC, first line treatment setting	Docetaxel, cisplatin, 5FU plus atezolizumab vs Docetaxel, cisplatin, 5FU	Yes

Figure 1. Key areas of anal cancer encompassed in this review