World Journal of Gastroenterology

TOPIC HIGHLIGHT

WJG 20th Anniversary Special Issues (20): Gastrointestinal surgery

Anal squamous cell carcinoma: An evolution in disease and management

Marc C Osborne, Justin Maykel, Eric K Johnson, Scott R Steele

Marc C Osborne, University of Minnesota, Minneapolis, MN 55415, United States

Justin Maykel, University of Massachusetts Memorial Medical Center, Worcester, MA 01609, United States

Eric K Johnson, Scott R Steele, Department of Surgery, Madigan Army Medical Center, Tacoma, WA 98431, United States

Author contributions: Osborne MC, Maykel J, Johnson EK, and Steele SR all contributed equally to this work; Osborne MC and Steele SR designed the review; Osborne MC, Maykel J, Johnson EK, and Steele SR contributed to the construction and critical revision of the paper.

Correspondence to: Scott R Steele, MD, FACS, FASCRS, Chief, 9040a Fitzsimmons Dr Department of Surgery, Madigan Army Medical Center, Tacoma, WA 98431, United States. harkersteele@mac.com

Telephone: +1-253-9682200 Fax: +1-253-9685900

Abstract

Anal cancer represents less than 1% of all new cancers diagnosed annually in the United States. Yet, despite the relative paucity of cases, the incidence of anal cancer has seen a steady about 2% rise each year over the last decade. As such, all healthcare providers need to be cognizant of the evaluation and treatment of anal squamous cell carcinoma. While chemoradiation remains the mainstay of therapy for most patients with anal cancer, surgery may still be required in recurrent, recalcitrant and palliative disease. In this manuscript, we will explore the diagnosis and management of squamous cell carcinoma of the anus.

Core tip: Despite advances in the diagnosis and management, we continue to see a steady rise in the incidence of anal squamous cell cancer. The management of anal cancer has evolved from mandatory surgery to sphincter preserving therapy and is now entering an era of screening and prevention. Chemoradiotherapy remains the primary therapy for anal cancer. Anal Pap smear and high-resolution anoscopy are emerging technologies for identification of precancerous lesions. A high index of suspicion and knowledge of the relevant anatomy and pathophysiology are essential to identify at risk group, avoid missed diagnosis, and provide proper counseling.

HISTORICAL PERSPECTIVE

Although rare, carcinoma of the anus remains a dreaded diagnosis (Figure 1). Fortunately, major advancements in oncology, both in the basic sciences as well as clinical therapeutics, have transformed the diagnosis and treatment of this disease. Up until the 1970's, the primary treatment for anal carcinoma was radical surgery with an abdominoperineal resection and permanent end colostomy. In May of 1974, Dr. Norman Nigro at Wayne State University published a series in Diseases of the Colon and Rectum of three patients with anal cancer treated with combined chemotherapy and radiation[1]. Two of the patients underwent surgical resection, and no remaining malignancy was identified in the specimens. The third

patient refused surgery and was disease free after 14 mo of follow up. Dr. Nigro's protocol of 3000 rads of external beam radiation, a five-day infusion of 5-fluorouricil, and a single injection of mitomycin-C became known as the "Nigro Protocol" and later signified a major advancement in the treatment of anal cancer, and in oncology in general. Further refinements would lead to combined chemotherapy and radiation as the primary treatment for anal carcinoma with most patients successfully treated without surgery.

We are currently are in the midst of another major shift in the treatment of anal cancer. Basic science and translational research have led to the identification of the virus which causes most cases of anal cancer and to a vaccine that can help prevent precursor lesions as well as tools to screen and treat high risk individuals. Current efforts are extending beyond treatment to screening and prevention. In this manuscript we will review the management of anal squamous cell carcinoma.

EPIDEMIOLOGY

When considered in the broader context, anal cancer is rare. It comprises about 2% of all gastrointestinal (GI) malignancies and only 0.4% of all new cancers diagnosed annually in the United States. The National Cancer Institute estimates that there were approximately 7000 new cases of anal cancer in the United States in 2013. The median age of diagnosis is 60 years old and there is a slightly higher incidence in women[2]. There is a very clear link between human papilloma virus (HPV) infection and anal cancer. HPV infects the skin and mucous membranes of the mouth, anus, penis and female reproductive tract. There are multiple known serotypes of HPV with varying degrees of oncogenicity. Serotypes 6 and 11 are most associated with anal and genital warts, while serotypes 16, 18, 31, 33, and 35 are more oncogenic and are associated with anal and cervical cancer. Population studies have demonstrated that 90% of patients with anal cancer are infected with either HPV 16 or 18[3]. Other known risk factors include a history of HPV related lesions-vulvar cancer, cervical cancer, vulvar or cervical dysplasia, anal or genital warts, a history of human immunodeficiency virus, smoking, men who have sex with men (MSM), and a history of transplantation with chronic immunosuppression. While anal cancer may occur in anyone, it is patients with one or more of these risk factors where focused effort is needed to make a tremendous difference in the incidence and outcomes of the disease.

PATHOLOGY

A clear distinction must be made between anal canal carcinoma and anal margin carcinoma, since the treatment can differ radically between the two. This requires some discussion of the anatomy of the anal canal. The anal canal is the last portion of the large intestine and extends

Figure 1 Anal squamous cell carcinoma (Courtesy of Richard Billingham, MD).

from the top of the anorectal ring to the anal verge. The anorectal ring is a structure where the levator ani muscles form a ring and are intimately associated with the top of the external anal sphincter muscles. The anal verge is the point where the anoderm, or the squamous epithelium of the anal canal distal to the dentate line, meets the hair-bearing perianal skin. The anal margin is the portion of the skin extending circumferentially 5 cm distal to the anal verge. The histology of tissue lining the anal canal is varied. At the top of the anal canal is the columnar epithelium similar to that seen in the rectum. Distally, the anal canal is lined by anoderm. As mentioned above the anoderm is stratified squamous epithelium. The anoderm is thinner than skin and lacks hair follicles or sebaceous glands. The easily identified line that marks the proximal most extent of the anoderm is called the dentate line. Just above the dentate line is a transition zone that may contain columnar, transitional, and stratified squamous epithelium. Noting the location of a tumor as either an anal canal *vs* anal margin lesion is important, as there significant differences in therapy between the two.

Lymphatic drainage proximal to the dentate line occurs along the rectum to the inferior mesenteric lymph nodes and to the nodes along the internal iliac vessels. Lymphatic drainage distal to the dentate line is primarily to the inguinal nodes. This comes into play both for the physical examination, as well as possible radiation portals during treatment.

By far the most common malignancy of the anal canal and anal margin is squamous cell carcinoma. This includes multiple histologies and descriptors that had previously been used including epidermoid, cloacogenic, mucoepidermoid, large-cell keratinizing, large-cell nonkeratinizing, and basaloid. Generally, the use of these terms has fallen out of use. Other malignancies of the anal canal and anal margin include adenocarcinoma, melanoma (Figure 2), gastrointestinal stromal tumors, neuroendocrine tumors, basal cell carcinoma, Paget's disease (intraepithelial adenocarcinoma), and verrucous carcinoma (Buschke-Lowenstein tumor).

Anal squamous cell carcinoma is thought to arise from a precursor lesion termed anal intraepithelial neoplasia

Figure 2 Anal melanoma (Courtesy of Richard Billingham, MD).

Figure 3 Anal intraepithelial neoplasia (Courtesy of Richard Billingham, MD).

Table 1 7th edition of the American joint Committee on Cancer TNM Staging			
Primary tumor (T)			
Tx	Primary tumor cannot be assessed		
Tis	Carcinoma *in situ*		
T1	Tumor < 2 cm in greatest dimension		
T2	Tumor between 2 and 5 cm in greatest dimension		
T3	Tumor > 5 cm in greatest dimension		
T4	Tumor invading adjacent organs		
Regional lymph nodes (N)			
Nx	Regional nodes cannot be assessed		
N0	No regional lymph node metastasis		
N1	Metastasis in the perirectal nodes		
N2	Metastasis in unilateral internal iliac and/or inguinal nodes		
N3	Metastasis bilateral internal iliac or inguinal nodes		
Distant metastasis (M)			
M0	No distant metastasis		
M1	Distant metastasis		
Stage			
0	Tis	N0	M0
I	T1	N0	M0
II	T2	N0	M0
	T3	N0	M0
IIIA	T1	N1	M0
	T2	N1	M0
	T3	N1	M0
	T4	N0	M0
IIIB	T4	N1	M0
	Any T	N2	M0
IV	Any T	N3	M0
	Any T	Any N	M1

(AIN) (Figure 3). AIN is a histologic, as opposed to cytologic, diagnosis with varying degrees of dysplasia. AIN I, II, and III have low, medium, and high grades of dysplasia respectively. There several terms used to classify AIN. Low-grade squamous intraepithelial lesion (LSIL) and high-grade intraepithelial lesion (HSIL) are commonly used. LSIL refers to AIN I and HSIL refers to AIN II or III. In subtle contrast low-grade anal intraepithelial neoplasia and high grade intraepithelial neoplasia have been proposed and refer to AIN I / II and AIN III. The natural history of AIN continues to be studied. Progression of low grades of AIN to high grade in HIV positive MSM or bisexual men is 62% at two years[4]. Progression of AIN to anal cancer will happen in 10% of cases with at least one year follow up[5].

STAGING

Staging for anal cancer is unlike the system used for malignancy in other portions of the bowel. Whereas malignancy in other portions of the bowel is staged according to depth of invasion, anal cancer is staged according to size. T1 lesions are less than 2 cm, T2 lesions are 2-5 cm and T3 lesions are greater than 5 cm. Lesions that invade vagina, urethra or bladder are classified as T4 regardless of size. Invasion to the rectum, skin, or sphincter muscles is no considered a T4 lesion. Additionally, lymph node metastasis is divided into perirectal, iliac and inguinal

lymph nodes. See Table 1 for the updated American Joint Committee on Cancer staging system for anal cancer.

SCREENING AND PREVENTION

Current efforts to prevent anal cancer are primarily directed at vaccination. There are two commonly available HPV vaccines. The first is a bivalent vaccine, HPV2 (Cervarix, GlaxoSmithKline) and has high efficacy against HPV 16 and 18. The quadrivalent vaccine, HPV4 (Gardasil, Merck & Co, Inc.) has high efficacy against HPV 6, 11, 16, and 18. The United States Centers for Disease Control recommend vaccination against HPV for both males and females ages 11-12 years. Females may be offered both the bivalent and quadrivalent vaccine. Males should be offered the quadrivalent vaccine. Additionally, CDC recommends vaccination of men who have sex with men up to age 26 years[6]. In a phase 3 randomized control trial of the quadrivalent vaccine, a subset analysis of men who have sex with men within the trial demonstrated significant prevention of precancerous lesions. Vaccine administration prevented development of AIN2 and AIN3 associated with HPV 6, 11, 16, and 18 in 50% of patients treated with the vaccine on intention to treat analysis[7,8].

No true screening exam for anal cancer exists, however anal pap smears, or high-resolution anoscopy are frequently used identify AIN. The scientific rationale for

Table 2 Treatment of anal intraepithelial neoplasia

Therapy	Pro	Con	Ref.
Watchful waiting	Avoids the morbidity of other therapies	Missed opportunity to potentially cure patient	[7]
	Low risk of interval development of carcinoma (for low-grade)	Need for close surveillance and reliable patient	
Topical imiquimod	Response rate of 48%-86%	Burning, irritation, variable patient compliance	[14-16]
		Recurrence or new lesions in untreated areas	
Topical 5-florouracil	High response rate, up to 90%	High recurrence rate, up to 50%	[17,18]
Wide local excision	Recurrence rates as low as 13% reported	Significant morbidity of anal stenosis, wound healing and incontinence	[19-21]
Targeted therapy with HRA	Evidence to prevent progression to anal cancer	High rate of persistent or recurrent disease in HIV positive patients	[22-24]
	Avoid anal stenosis and incontinence		

HRA: High-resolution anoscopy; HIV: Human immunodeficiency virus.

an anal pap smear is derived from the similar pathogenesis between cervical and anal cancer. Similar to a cervical pap smear, an anal pap smear is obtained by inserting a Dacron swab into the anal canal to a level 1-2 cm above the dentate line. The anal canal is then circumferentially swabbed and either plated or placed in formaldehyde. The cells are then stained with the Papanicolau stain to identify dysplastic cells.

High-resolution anoscopy (HRA) applies the techniques of colposcopy, used to detect pre-cancerous lesions in the cervix, to identify pre-cancerous anal lesions. 3% acetic acid and Lugol's iodine solution is applied to the anal canal. The anal canal is then thoroughly inspected with a high resolution microscope and HPV infected cells are then easily seen as characteristic white lesions[9].

The exact method for screening any given population at risk has not been well described. While HRA has a high sensitivity and specificity, it is not widely available. Anal Pap smear is easy to perform, but has a sensitivity and specificity of 69%-93% and 32%-59% respectively when compared to HRA. Anal Pap smear has a high false-negative rate of 23% of HIV negative MSM and 45% for HIV-positive MSM[10-13].

There are several treatment options once AIN is discovered. These include watchful waiting, topical therapy (*i.e.*, 5-FU, imiquimod), photodynamic therapy, cryotherapy, and focal destruction (surgery/ablation). Table 2 summarizes the pros and cons of the various treatment options. It is important to note that treatment of AIN remains controversial. Overriding the diverging viewpoint in treatment is the (as of yet) unknown degree of progression in those with low-grade AIN, especially in immunocompetent individuals. On the other hand, advocates for a "watchful waiting" approach cite the need to avoid the morbidity associated with repeated focal destruction (stenosis, wound healing problems, chronic pain). In between, a high rate of clearance can be achieved through the use of HRA with targeted biopsies and directed therapy.

Alternatively, high-risk cohorts such as HIV-positive patients and MSM have demonstrated higher rates of progression to invasion, as well as increased rates of recurrence even after aggressive attempts at eradication. This has resulted in some experts suggesting that leading

some to suggest expectant management in these patients with surveillance every 4-6 mo[7].

Several topical therapies have been approved for the treatment of AIN with varying degrees of success[14-26]. Imiquimod is an immune response modifier that has both anti-HPV and anti-tumor properties. The use of topical 5% imiquimod cream has a reported response rate from 48%-86%[14]. Side effects range from local irritation and burning to skin erosions, often leading to decreased compliance. In addition, local recurrence rates remain high, resulting in a need for close long-term follow-up[15]. Topical 5-FU typically requires a treatment period of 9-16 wk, with clinical response rates have been reported up to 90%. However, recurrence rates remain as high as 50%[16]. Side effects are mild (but common), and include local skin irritation and hypo-pigmentation. Finally, the use of photosensitizing agents followed by photodynamic therapy has been described in patients with AIN; however, studies are limited and its effectiveness is yet to be determined[17].

Targeted destruction of AIN with appropriate follow-up is another accepted treatment option and represents a more aggressive therapeutic approach. Several techniques have been described including wide local excision (WLE) with 1 cm margins. This is classically guided by frozen section through the use of 4-quadrant anal mapping with biopsies-performed with ever expanding circles out from the anus. Downsides to this procedure include the need for skin grafting or mobilization of local flaps to cover large skin and mucosal defects following WLE. In addition, total excision of all disease is difficult. Recurrence rates have been reported in 13%-63% of patients[18]. Additionally, this approach carries significant risk of local wound complications such as stenosis and incontinence.

HRA guided cautery ablation has been shown to be effective in destroying AIN without the morbidity associated with WLE. In some series, it has also demonstrated the ability to prevent progression to invasive cancer. However, like other attempts at disease eradication, high rates of persistent or recurrent disease (up to 80%) remains a problem, especially among HIV-positive patients, immunosuppressed and MSM patients[19].

PRESENTATION AND EVALUATION

Twenty percent of patients with anal cancer will have no symptoms highlighting the need for screening and prevention. Other symptoms include transanal bleeding and anal pain. Diagnosis is made with physical examination and biopsy, which may be done in the office or in the operating room. Patients should be fully staged with a colonoscopy and computed tomography scan of the chest, abdomen, and pelvis. Women should undergo a gynecologic exam and cervical cancer screening. Additional studies can include positron emission tomography (PET)-CT, endoanal ultrasound, and MRI of the pelvis. The routine use of these additional studies is controversial. Endoanal ultrasound can easily identify the depth of invasion of a lesion, particularly with respect to involvement of the anal sphincters. MRI can be useful in evaluating pelvic lymphadenopathy as well as depth of the tumor. Palpable or suspected inguinal lymph nodes should be evaluated with fine needle aspiration. PET CT will change the stage of the lesion in up to 20% of cases and is recommended for planning of chemoradiation in the latest version of the National Comprehensive Cancer Network Guidelines[8].

TREATMENT

Treatment of anal cancer is dependent on location and stage. Anal canal cancer is primarily treated with chemoradiation. Since Dr. Nigro's protocol was reported several iterations of therapy have been explored. Chemotherapy with radiation *vs* radiation alone has been evaluated in multicenter, prospective, randomized controlled trials[26]. These trials demonstrate a significant improvement in local recurrence as well need for colostomy in combined chemoradiotherapy *vs* radiotherapy alone. There were nearly 50% fewer failures of local control seen with combined chemoradiotherapy *vs* radiotherapy alone in the multicenter UK trial. No improvement in overall survival was seen in either major trial[27-29]. The role of cisplatin has also been evaluated in multicenter, prospective randomized controlled trials. A major American trial reported in 2008 evaluated 644 patients and compared a standard group treated with radiotherapy and concurrent 5-FU and mitomycin C *vs* radiotherapy and concurrent 5-FU with cisplatin. Rates of 5-year disease free survival, overall survival, local recurrence, and distant metastasis were similar between the groups. There was a higher rate of creation of a colostomy in the cisplatin group, but more severe hematologic toxicities were seen with the mitomycin C group[30]. The recently reported multicenter ACT Ⅱ trial from the UK also demonstrated no difference in response rates or in survival between patients receiving mitomycin C *vs* cisplatin. This trial notably reported a high complete response rate of 90% at 26 wk post chemotherapy[31]. Current NCCN guidelines recommend 5-FU with mitomycin C and concurrent radiotherapy for all localized anal canal carcinomas. 5-FU with cisplatin is the recommended therapy for widely metastatic disease[8].

Toxicity is common with combined chemoradiotherapy for anal cancer and a break or pause in treatment is seen 40%-60% of the time. Pooled analysis has shown that avoiding breaks in therapy, either planned or unplanned, results in improved local control and lower rates of colostomy formation[32-36]. Intensity modulated radiation therapy is a technique that allows for more precise mapping of the radiation field based on data obtained from cross sectional imaging such as CT or MRI. This technique is associated with lower rates of toxicity with similar efficacy as compared to standard external beam radiation[37,38]. Initial radiation fields include the pelvis and the inguinal lymph nodes. The field may be narrowed at predefined points in the treatment course to exclude the inguinal lymph nodes in node negative patients or to provide a boost to larger tumors.

Anal margin cancers are treated in a slightly different fashion from anal canal tumors. Early stage, T1 and early T2, anal margin cancers that do not involve the sphincter muscle can be treated with wide local excision with 1 cm margins. Five-year survival rates of up to 88% have been reported with wide local excision alone for these small tumors. Larger tumors and patients with positive nodes should be treated in a manner similar to anal canal cancer with chemoradiotherapy.

SURVEILLANCE, OUTCOMES AND SALVAGE TREATMENT

Initial follow up should be 8-12 after finishing chemoradiotherapy. Patients are then classified as complete remission, persistent disease, and progressive disease. 10%-30% of patients will have persistent or recurrent disease after chemoradiotherapy. Risk factors for persistent or recurrent disease are positive HIV status, high T and N stage at original presentation, and inability to complete chemoradiotherapy[31,39,40]. Progressive disease is biopsied and restaged. Persistent disease should be reexamined in one month. Disease that persists and does not progress, and does not regress, can be followed without surgical therapy up to 6 mo post chemoradiotherapy. Follow up from the large ACT Ⅱ UK trial demonstrated that 29% of patients with persistent disease at 11 wk post chemoradiotherapy, were disease free at 26 wk. If during close follow up there is progression of disease or persistent disease at 6 mo post chemoradiotherapy, the patient should be biopsied and restaged.

Patients who achieve complete remission should be followed every three to six months for five years. Digital rectal exam, anoscopy, and groin examination should be documented. A CT of the chest, abdomen, and pelvis should be done annually for 3 years in T, T4, or N+ tumors.

RECURRENT DISEASE

Surgical therapy is the main treatment for patients who have a local recurrence or who do not achieve a complete

response with chemoradiotherapy. Often referred to as a salvage APR, the results of this operation are modest. Salvage APR results in five-year local-regional control of 30%-77% of patients[39,41,42]. Wound complications are common, up to 80%, after chemoradiotherapy for anal cancer and consideration should be given to a flap type reconstruction of the perineum[43].

METASTATIC DISEASE AND TARGETED THERAPY

Metastatic anal cancer is normally treated with systemic chemotherapy, typically utilizing a combination of 5-FU and cisplatin. In general, there is not much role for surgery in the setting of metastatic disease except to provide palliation for pain, bleeding, or fecal incontinence. Overall, about 60% one-year survival and 32% 5-year survival rates with systemic chemotherapy have been reported[44].

On the positive side, there is increasing interest in newer, targeted therapies for anal cancer. It was recently demonstrated that there is a high rate of expression of epidermal growth factor receptor (EGFR) within most anal cancer cell lines[45]. Cetuximab is an EGFR blocker that is used along with radiotherapy to treat squamous cell carcinoma of the head and neck. There have been two small early phase trials to evaluate the use of cetuximab in anal cancer[46,47]. Both trials demonstrated significant toxicities with cetuximab that precluded incorporation of this agent into routine chemotherapy protocols for anal cancer. One of the trials demonstrated a 95% initial complete response rate, with a 3-year local regional control rate of 64.2%. Therapy targeted against the EGFR may ultimately prove beneficial in the future with less toxic agents; however, the lack of widespread data at this stage precludes definitive recommendations regarding its role.

Another type of therapy that has been used is intensity-modulated radiation therapy-a type of high precision radiation therapy. The primary benefit of this modality is the ability to create a more precise and complex radiation field, and thus avoiding radiation toxicity to normal surrounding structures and allow for dose escalation. Current literature is limited to single institution series with non-randomized studies that provide conflicting results[48,49]. Data from a phase 2 Radiation Therapy Oncology Group trial demonstrated two-year local control, overall survival, colostomy-free survival, and metastasis-free survival rates of 95%, 94%, 90%, and 92%, respectively[50]. Again, further use with long-term results in greater number of patients will provide more insight as to its place in the therapeutic regimen.

CONCLUSION

Anal squamous cell carcinoma is a rare malignancy and is highly associated with the HPV virus. A high index of suspicion and familiarity with the relevant anatomy is required for accurate diagnosis. While the primary therapy is chemoradiation, surgery has a role in the treatment of persistent or recurrent disease. Anal Pap smear and high-resolution anoscopy are emerging as useful screening tools to identify precancerous lesions. Vaccination against HPV has been shown to prevent progression of precancerous lesions in high-risk individuals.

REFERENCES

1   **Nigro ND**, Vaitkevicius VK, Considine B. Combined therapy for cancer of the anal canal: a preliminary report. *Dis Colon Rectum* 1974; **17**: 354-356 [PMID: 4830803 DOI: 10.1007/BF02586980]

2   Anal Cancer [Internet]. U.S. Natl. Institutes Heal. Available from: URL: http://www.cancer.gov/cancertopics/types/anal. Accessed on Feb 3, 2014

3   **Parkin DM**, Bray F. Chapter 2: The burden of HPV-related cancers. *Vaccine* 2006; **24** Suppl 3: S3/11-S3/25 [PMID: 16949997]

4   **Palefsky JM**, Holly EA, Hogeboom CJ, Ralston ML, DaCosta MM, Botts R, Berry JM, Jay N, Darragh TM. Virologic, immunologic, and clinical parameters in the incidence and progression of anal squamous intraepithelial lesions in HIV-positive and HIV-negative homosexual men. *J Acquir Immune Defic Syndr Hum Retrovirol* 1998; **17**: 314-319 [PMID: 9525431 DOI: 10.1097/00042560-199804010-00004]

5   **Devaraj B**, Cosman BC. Expectant management of anal squamous dysplasia in patients with HIV. *Dis Colon Rectum* 2006; **49**: 36-40 [PMID: 16283561 DOI: 10.1007/s10350-005-0229-z]

6   Recommendations on the Use of Quadrivalent Human Papillomavirus Vaccine in Males-Advisory Committee on Immunization Practices (ACIP), 2011 MMWR. December 23, 2011; 60: 1705-1708. Available from: URL: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6050a3.htm. Accessed on Feb 26, 2014)

7   **Palefsky JM**, Giuliano AR, Goldstone S, Moreira ED, Aranda C, Jessen H, Hillman R, Ferris D, Coutlee F, Stoler MH, Marshall JB, Radley D, Vuocolo S, Haupt RM, Guris D, Garner EI. HPV vaccine against anal HPV infection and anal intraepithelial neoplasia. *N Engl J Med* 2011; **365**: 1576-1585 [PMID: 22029979 DOI: 10.1056/NEJMoa1010971]

8   NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines) Anal Carcinoma Version 2. 2014. Available from: URL: http://www.nccn.org/professionals/physician_gls/pdf/anal.pdf. Accessed on Feb 26, 2014

9   **Chang GJ**, Berry JM, Jay N, Palefsky JM, Welton ML. Surgical treatment of high-grade anal squamous intraepithelial lesions: a prospective study. *Dis Colon Rectum* 2002; **45**: 453-458 [PMID: 12006924 DOI: 10.1007/s10350-004-6219-8]

10   **Arain S**, Walts AE, Thomas P, Bose S. The Anal Pap Smear: Cytomorphology of squamous intraepithelial lesions. *Cytojournal* 2005; **2**: 4 [PMID: 15715910 DOI: 10.1186/1742-6413-2-4]

11   **Palefsky JM**, Holly EA, Hogeboom CJ, Berry JM, Jay N, Darragh TM. Anal cytology as a screening tool for anal squamous intraepithelial lesions. *J Acquir Immune Defic Syndr Hum Retrovirol* 1997; **14**: 415-422 [PMID: 9170415 DOI: 10.1097/00042560-199704150-00004]

12   **Fox PA**, Seet JE, Stebbing J, Francis N, Barton SE, Strauss S, Allen-Mersh TG, Gazzard BG, Bower M. The value of anal cytology and human papillomavirus typing in the detection of anal intraepithelial neoplasia: a review of cases from an anoscopy clinic. *Sex Transm Infect* 2005; **81**: 142-146 [PMID: 15800092 DOI: 10.1136/sti.2003.008318]

13   **Chin-Hong PV**, Berry JM, Cheng SC, Catania JA, Da Costa M, Darragh TM, Fishman F, Jay N, Pollack LM, Palefsky JM. Comparison of patient- and clinician-collected anal cytology samples to screen for human papillomavirus-associated anal

intraepithelial neoplasia in men who have sex with men. *Ann Intern Med* 2008; **149**: 300-306 [PMID: 18765699 DOI: 10.7326/0003-4819-149-5-200809020-00004]

14 **Wieland U**, Brockmeyer NH, Weissenborn SJ, Hochdorfer B, Stücker M, Swoboda J, Altmeyer P, Pfister H, Kreuter A. Imiquimod treatment of anal intraepithelial neoplasia in HIV-positive men. *Arch Dermatol* 2006; **142**: 1438-1444 [PMID: 17116834 DOI: 10.1001/archderm.142.11.1438]

15 **Kreuter A**, Potthoff A, Brockmeyer NH, Gambichler T, Stücker M, Altmeyer P, Swoboda J, Pfister H, Wieland U. Imiquimod leads to a decrease of human papillomavirus DNA and to a sustained clearance of anal intraepithelial neoplasia in HIV-infected men. *J Invest Dermatol* 2008; **128**: 2078-2083 [PMID: 18273049 DOI: 10.1038/jid.2008.24]

16 **Richel O**, Wieland U, de Vries HJ, Brockmeyer NH, van Noesel C, Potthoff A, Prins JM, Kreuter A. Topical 5-fluorouracil treatment of anal intraepithelial neoplasia in human immunodeficiency virus-positive men. *Br J Dermatol* 2010; **163**: 1301-1307 [PMID: 20716208 DOI: 10.1111/j.1365-2133.2010.09982.x]

17 **Kruijt B**, van der Snoek EM, Sterenborg HJ, Amelink A, Robinson DJ. A dedicated applicator for light delivery and monitoring of PDT of intra-anal intraepithelial neoplasia. *Photodiagnosis Photodyn Ther* 2010; **7**: 3-9 [PMID: 20230986 DOI: 10.1016/j.pdpdt.2010.01.006]

18 **Margenthaler JA**, Dietz DW, Mutch MG, Birnbaum EH, Kodner IJ, Fleshman JW. Outcomes, risk of other malignancies, and need for formal mapping procedures in patients with perianal Bowen's disease. *Dis Colon Rectum* 2004; **47**: 1655-160; discussion 1655-160; [PMID: 15540295]

19 **Chang GJ**, Welton ML. Human papillomavirus, condylomata acuminata, and anal neoplasia. *Clin Colon Rectal Surg* 2004; **17**: 221-230 [PMID: 20011263 DOI: 10.1055/s-2004-836942]

20 **Rosen T**, Harting M, Gibson M. Treatment of Bowen's disease with topical 5% imiquimod cream: retrospective study. *Dermatol Surg* 2007; **33**: 427-31; discussion 431-2 [PMID: 17430376 DOI: 10.1111/j.1524-4725.2007.33089.x]

21 **Graham BD**, Jetmore AB, Foote JE, Arnold LK. Topical 5-fluorouracil in the management of extensive anal Bowen's disease: a preferred approach. *Dis Colon Rectum* 2005; **48**: 444-450 [PMID: 15747068 DOI: 10.1007/s10350-004-0901-8]

22 **Brown SR**, Skinner P, Tidy J, Smith JH, Sharp F, Hosie KB. Outcome after surgical resection for high-grade anal intraepithelial neoplasia (Bowen's disease). *Br J Surg* 1999; **86**: 1063-1066 [PMID: 10460644 DOI: 10.1046/j.1365-2168.1999.01184.x]

23 **Marchesa P**, Fazio VW, Oliart S, Goldblum JR, Lavery IC. Perianal Bowen's disease: a clinicopathologic study of 47 patients. *Dis Colon Rectum* 1997; **40**: 1286-1293 [PMID: 9369101 DOI: 10.1007/BF02050810]

24 **Goldstone SE**, Kawalek AZ, Huyett JW. Infrared coagulator: a useful tool for treating anal squamous intraepithelial lesions. *Dis Colon Rectum* 2005; **48**: 1042-1054 [PMID: 15868241 DOI: 10.1007/s10350-004-0889-0]

25 **Watson AJ**, Smith BB, Whitehead MR, Sykes PH, Frizelle FA. Malignant progression of anal intra-epithelial neoplasia. *ANZ J Surg* 2006; **76**: 715-717 [PMID: 16916390 DOI: 10.1111/j.1445-2197.2006.03837.x]

26 **Steele SR**, Varma MG, Melton GB, Ross HM, Rafferty JF, Buie WD. Practice parameters for anal squamous neoplasms. *Dis Colon Rectum* 2012; **55**: 735-749 [PMID: 22706125 DOI: 10.1097/DCR.0b013e318255815e]

27 Epidermoid anal cancer: results from the UKCCCR randomised trial of radiotherapy alone versus radiotherapy, 5-fluorouracil, and mitomycin. UKCCCR Anal Cancer Trial Working Party. UK Co-ordinating Committee on Cancer Research. *Lancet* 1996; **348**: 1049-1054 [PMID: 8874455 DOI: 10.1016/S0140-6736(96)03409-5]

28 **Bartelink H**, Roelofsen F, Eschwege F, Rougier P, Bosset JF, Gonzalez DG, Peiffert D, van Glabbeke M, Pierart M. 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**: 2040-2049 [PMID: 9164216]

29 **Ajani JA**, Winter KA, Gunderson LL, Pedersen J, Benson AB, Thomas CR, Mayer RJ, Haddock MG, Rich TA, Willett C. Fluorouracil, mitomycin, and radiotherapy vs fluorouracil, cisplatin, and radiotherapy for carcinoma of the anal canal: a randomized controlled trial. *JAMA* 2008; **299**: 1914-1921 [PMID: 18430910 DOI: 10.1001/jama.299.16.1914]

30 **James RD**, Glynne-Jones R, Meadows HM, Cunningham D, Myint AS, Saunders MP, Maughan T, McDonald A, Essapen S, Leslie M, Falk S, Wilson C, Gollins S, Begum R, Ledermann J, Kadalayil L, Sebag-Montefiore D. 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**: 516-524 [PMID: 23578724 DOI: 10.1016/S1470-2045(13)70086-X]

31 **Ben-Josef E**, Moughan J, Ajani JA, Flam M, Gunderson L, Pollock J, Myerson R, Anne R, Rosenthal SA, Willett C. Impact of overall treatment time on survival and local control in patients with anal cancer: a pooled data analysis of Radiation Therapy Oncology Group trials 87-04 and 98-11. *J Clin Oncol* 2010; **28**: 5061-5066 [PMID: 20956625 DOI: 10.1200/JCO.2010.29.1351]

32 **Hannoun-Levi JM**, Ortholan C, Resbeut M, Teissier E, Ronchin P, Cowen D, Zaccariotto A, Bénézery K, François E, Salem N, Ellis S, Azria D, Gerard JP. High-dose split-course radiation therapy for anal cancer: outcome analysis regarding the boost strategy (CORS-03 study). *Int J Radiat Oncol Biol Phys* 2011; **80**: 712-720 [PMID: 20619552 DOI: 10.1016/j.ijrobp.2010.02.055]

33 **Widder J**, Kastenberger R, Fercher E, Schmid R, Langendijk JA, Dobrowsky W, Pötter R. Radiation dose associated with local control in advanced anal cancer: retrospective analysis of 129 patients. *Radiother Oncol* 2008; **87**: 367-375 [PMID: 18501453 DOI: 10.1016/j.radonc.2008.05.001]

34 **Glynne-Jones R**, Sebag-Montefiore D, Adams R, McDonald A, Gollins S, James R, Northover JM, Meadows HM, Jitlal M. "Mind the gap"--the impact of variations in the duration of the treatment gap and overall treatment time in the first UK Anal Cancer Trial (ACT I). *Int J Radiat Oncol Biol Phys* 2011; **81**: 1488-1494 [PMID: 20934265 DOI: 10.1016/j.ijrobp.2010.07.1995]

35 **Janssen S**, Meier zu Eissen J, Kolbert G, Bremer M, Karstens JH, Meyer A. Anal cancer treated with radio-chemotherapy: correlation between length of treatment interruption and outcome. *Int J Colorectal Dis* 2009; **24**: 1421-1428 [PMID: 19649642 DOI: 10.1007/s00384-009-0775-2]

36 **Salama JK**, Mell LK, Schomas DA, Miller RC, Devisetty K, Jani AB, Mundt AJ, Roeske JC, Liauw SL, Chmura SJ. Concurrent chemotherapy and intensity-modulated radiation therapy for anal canal cancer patients: a multicenter experience. *J Clin Oncol* 2007; **25**: 4581-4586 [PMID: 17925552 DOI: 10.1200/JCO.2007.12.0170]

37 **Pepek JM**, Willett CG, Wu QJ, Yoo S, Clough RW, Czito BG. Intensity-modulated radiation therapy for anal malignancies: a preliminary toxicity and disease outcomes analysis. *Int J Radiat Oncol Biol Phys* 2010; **78**: 1413-1419 [PMID: 20231064 DOI: 10.1016/j.ijrobp.2009.09.046]

38 **Roohipour R**, Patil S, Goodman KA, Minsky BD, Wong WD, Guillem JG, Paty PB, Weiser MR, Neuman HB, Shia J, Schrag D, Temple LK. Squamous-cell carcinoma of the anal canal: predictors of treatment outcome. *Dis Colon Rectum* 2008; **51**: 147-153 [PMID: 18180997 DOI: 10.1007/s10350-007-9125-z]

39 **Papaconstantinou HT**, Bullard KM, Rothenberger DA, Madoff RD. Salvage abdominoperineal resection after failed Nigro protocol: modest success, major morbidity. *Colorec-*

tal Dis 2006; **8**: 124-129 [PMID: 16412072 DOI: 10.1111/j.1463-1318.2005.00911.x]

40 **Glynne-Jones R**. James R, Meadows H, Begum R, Cunningham D, Northover J, Ledermann JA, Beare S, Kadalayil L, Sebag-Montefiore D. Optimum time to assess complete clinical response (CR) following chemoradiation (CRT) using mitomycin (MMC) or cisplatin (CisP), with or without maintenance CisP/5FU in squamous cell carcinoma of the anus: Results of ACT II. 2012 ASCO Annual Meeting Abstracts. J Clin Oncol, Vol 30, No 15_suppl (May 20 Supplement), 2012: 4004. Available from: URL: http://meeting.ascopubs.org/cgi/content/abstract/30/15_suppl/4004

41 **van der Wal BC**, Cleffken BI, Gulec B, Kaufman HS, Choti MA. Results of salvage abdominoperineal resection for recurrent anal carcinoma following combined chemoradiation therapy. *J Gastrointest Surg* 2001; **5**: 383-387 [PMID: 11985979]

42 **Ghouti L**, Houvenaeghel G, Moutardier V, Giovannini M, Magnin V, Lelong B, Bardou VJ, Delpero JR. Salvage abdominoperineal resection after failure of conservative treatment in anal epidermoid cancer. *Dis Colon Rectum* 2005; **48**: 16-22 [PMID: 15690652 DOI: 10.1007/s10350-004-0746-1]

43 **Chessin DB**, Hartley J, Cohen AM, Mazumdar M, Cordeiro P, Disa J, Mehrara B, Minsky BD, Paty P, Weiser M, Wong WD, Guillem JG. Rectus flap reconstruction decreases perineal wound complications after pelvic chemoradiation and surgery: a cohort study. *Ann Surg Oncol* 2005; **12**: 104-110 [PMID: 15827789 DOI: 10.1245/ASO.2005.03.100]

44 **Faivre C**, Rougier P, Ducreux M, Mitry E, Lusinchi A, Lasser P, Elias D, Eschwege F. [5-fluorouracile and cisplatinum combination chemotherapy for metastatic squamous-cell anal cancer]. *Bull Cancer* 1999; **86**: 861-865 [PMID: 10572237]

45 **Paliga A**, Onerheim R, Gologan A, Chong G, Spatz A, Niazi T, Garant A, Macheto D, Alcindor T, Vuong T. EGFR and K-ras gene mutation status in squamous cell anal carcinoma: a role for concurrent radiation and EGFR inhibitors? *Br J Cancer* 2012; **107**: 1864-1868 [PMID: 23093229 DOI: 10.1038/bjc.2012.479]

46 **Deutsch E**, Lemanski C, Pignon JP, Levy A, Delarochefordiere A, Martel-Lafay I, Rio E, Malka D, Conroy T, Miglianico L, Becouarn Y, Malekzadeh K, Paris E, Juzyna B, Ezra P, Azria D. Unexpected toxicity of cetuximab combined with conventional chemoradiotherapy in patients with locally advanced anal cancer: results of the UNICANCER ACCORD 16 phase II trial. *Ann Oncol* 2013; **24**: 2834-2838 [PMID: 24026540 DOI: 10.1093/annonc/mdt368]

47 **Olivatto LO**, Vieira FM, Pereira BV, Victorino AP, Bezerra M, Araujo CM, Erlich F, Faroni L, Castro L, Lusis EC, Marins A, Ferreira CG. Phase 1 study of cetuximab in combination with 5-fluorouracil, cisplatin, and radiotherapy in patients with locally advanced anal canal carcinoma. *Cancer* 2013; **119**: 2973-2980 [PMID: 23674135 DOI: 10.1002/cncr.28045]

48 **Saarilahti K**, Arponen P, Vaalavirta L, Tenhunen M. The effect of intensity-modulated radiotherapy and high dose rate brachytherapy on acute and late radiotherapy-related adverse events following chemoradiotherapy of anal cancer. *Radiother Oncol* 2008; **87**: 383-390 [DOI: 10.1016/j.radonc.2008.04.011]

49 **Dasgupta T**, Rothenstein D, Chou JF, Zhang Z, Wright JL, Saltz LB, Temple LK, Paty PB, Weiser MR, Guillem JG, Nash GM, Goodman KA. Intensity-modulated radiotherapy vs. conventional radiotherapy in the treatment of anal squamous cell carcinoma: a propensity score analysis. *Radiother Oncol* 2013; **107**: 189-194 [PMID: 23692961 DOI: 10.1016/j.radonc.2013.03.012]

50 **Kachnic LA**, Tsai HK, Coen JJ, Blaszkowsky LS, Hartshorn K, Kwak EL, Willins JD, Ryan DP, Hong TS. Dose-painted intensity-modulated radiation therapy for anal cancer: a multi-institutional report of acute toxicity and response to therapy. *Int J Radiat Oncol Biol Phys* 2012; **82**: 153-158 [PMID: 21095071 DOI: 10.1016/j.ijrobp.2010.09.030]

Baishideng®