

Treatment of the Primary Tumor in Anal Canal Cancers



Rob Glynn-Jones, FRCR, FRCP^{a,*}, Sheela Rao, MD, FRCP^{b,c}

KEYWORDS

- Squamous cell carcinoma of the anus • Anal carcinoma • Chemotherapy
- Radiotherapy • Chemoradiation • Combined modality • Immunotherapy
- Local recurrence

KEY POINTS

- Squamous cell carcinoma of the anus is generally a localized disease with a relatively low risk of metastatic disease at presentation; thus, local control is the overriding aim of treatment.
- Randomized phase III trials have established the combination of 5-fluorouracil-based chemoradiation concurrent with mitomycin C as the standard of care rather than primary surgery.
- The TNM clinical staging system is based on accurate assessment of size (T stage), regional lymph node involvement, and metastatic spread.
- Assessment and management of anal cancer are best determined by specialist multidisciplinary teams, and treatment should be carried out in specialized centers.
- Future research should attempt to integrate novel biomarker-driven targets, such as anti-CTLA4, anti-programmed cell death, and programmed cell death-ligand 1, into chemoradiation schedules.

INTRODUCTION

Squamous cell carcinoma of the anus (SCCA) is an uncommon malignancy representing approximately 2% of all gastrointestinal malignancies. The incidence has been increasing over the past decade, probably reflecting more widespread infection with the main causal factor human papillomavirus (HPV).

Conflict of Interest Statements: R. Glynn-Jones has received honoraria for lectures and advisory boards and has been supported in attending international meetings by Merck, Pfizer, Sanofi-Aventis, Eli-Lilly, and Roche. He has also received unrestricted grants for research from Merck Serono, Sanofi-Aventis, and Roche. S. Rao has received honoraria for lectures and advisory boards and has been supported in attending international meetings by Merck Serono, Roche, Sanofi-Aventis, Eli Lilly, Servier, Amgen, and Celgene.

^a Mount Vernon Centre for Cancer Treatment, Rickmansworth Road, Northwood, Middlesex HA6 2RN, UK; ^b Royal Marsden Hospital, London, UK; ^c Royal Marsden Hospital, Downs Road, Sutton, Surrey SM2 5PT, UK

* Corresponding author.

E-mail address: Rob.glynnjones@nhs.net

Surg Oncol Clin N Am 26 (2017) 73–90
<http://dx.doi.org/10.1016/j.soc.2016.07.003>

1055-3207/17/© 2016 Elsevier Inc. All rights reserved.

surgonc.theclinics.com

SCCA is generally a localized disease with a low risk of metastatic disease at presentation. Retrospective studies and randomized trials suggest that locoregional failure is the predominant pattern of relapse,¹⁻⁴ usually in the radiotherapy high-dose volume, and often within the first 2 years following completion of chemoradiation (CRT) treatment. Uncontrolled local recurrence is ultimately responsible for most cancer-related deaths, making local control the primary aim of treatment.

Surgical resection was the standard treatment in the 1970s, which involved removal of the anal canal and a permanent stoma. In the past, radiation alone was also often used to treat SCCA using high doses with split-course schedules and interstitial brachytherapy. The pioneering work of Nigro and colleagues^{5,6} and subsequent confirmatory studies in the United States highlighted the efficacy of CRT using relatively low doses of fractionated radiotherapy (30–45 Gy) combined with 5-fluorouracil (5-FU) and mitomycin C (MMC). Subsequently, 2 randomized trials^{7,8} compared a radiotherapy schedule of 45 Gy boosted with a further 15 to 25 Gy after a gap of 6 weeks against an identical regimen with concurrent 5-FU/MMC. These trials showed radiation alone could result in local control in approximately 45% to 55% of patients. However, both trials confirmed chemoradiotherapy significantly improved outcomes over radiation alone.

Significant toxicity was reported for MMC. So concurrent 5-FU and MMC or 5-FU alone were randomly compared in the CRT component in the RTOG 8704 trial.⁹ The addition of MMC significantly improved both disease-free survival (DFS) and colostomy-free survival (CFS).⁹ Thus, a series of randomized trials⁷⁻¹¹ all confirmed the efficacy of concurrent CRT with 5-FU/MMC and relegated the role of surgery to salvage of CRT failures. The small Action Clinique Coordonees en Cancerologie Digestive (ACCORD-03) trial, in contrast, used concurrent 5-FU/cisplatin.¹²

The standard of care both in Europe and North America is 5-FU/MMC CRT and is recommended in guidelines.^{13,14} This schedule results in complete tumor regression in 80% to 90%, with a high level of permanent disease control particularly for cT1/T2 tumors. The 5-year overall survival (OS) reached 78% in the MMC arm of the RTOG 9811 trial,¹⁵ 71% in the CRT-alone arms without neoadjuvant chemotherapy (NACT) in ACCORD-03,¹² and 79% in the MMC arm of Anal Cancer Trial II (ACT II).¹¹

Preservation of sphincter function is usually achieved; but with doses of 50 to 60 Gy, there is a risk of fecal incontinence.¹⁶ In more advanced T3/T4 cancers with nodal metastases, it is more difficult to achieve local control¹⁷; a substantial proportion of such patients will fail within 2 years. In the ACT II, patients with cT3/T4 cancers and nodal metastases had a 3-year progression-free survival (PFS) of 63%.¹¹

Randomized phase III trials by RTOG 9811,¹⁰ the ACCORD-03 phase III trial,¹² and the ACT II trial¹¹ failed to show any additional benefit in terms of PFS/DFS by increasing the radiotherapy boost dose or replacing MMC with cisplatin during CRT. Additional cisplatin-based chemotherapy given as induction before CRT^{10,12} or as maintenance or consolidation after CRT¹¹ has not improved outcomes (Table 1).

SCCA regresses slowly following radiation or CRT. In early trials a 6 to 8 week planned gap between the completion of CRT and a radiotherapy boost allowed the acute toxicity of skin and mucosal surfaces to resolve.^{7,8} During this interval the tumor would shrink, and permit an interstitial implant to the smallest possible volume - minimizing the risk of radiation induced necrosis. This strategy also allowed selection of nonresponders for salvage either by dose-escalation of the radiotherapy boost or by surgical resection. Later trials continued this approach although shortened the interval.^{10,12} However this practice defied radiobiological principles, because the gap

Table 1

The more recent phase III randomized trials (1998–2008) in squamous cell carcinoma of the anus and their characteristics of the randomized trials comparing different chemoradiation regimens

Trial Name (Years)	Number of Patients	Design	RT Dose	Comparison 1	Comparison 2	Primary End Point
RTOG 9811 (1998–2005) (Ajani et al, ¹⁰ 2008)	644	NACT cisplatin/5-FU Then 5-FU/cisplatin/RT (ie, 4 courses) vs 5-FU/ MMC/RT	Phase I 45 Gy/25# in 5.0–6.5 wk T3/T4, N+, or residual T2 boost to 54–59 Gy	NACT with 5-FU 1000 mg/m ² days 1–4, 29–32, and cisplatin 75 mg/m ² then CRT 5- FU/cisplatin vs MMC 10 mg/m ² days 1 and 29 and 5-FU 1000 mg/ m ² days 1–4, 29–32 CRT		DFS
ACCORD-03 (1999–2005) (Peiffert et al, ¹² 2012)	307	2 × 2 factorial NACT (5-FU/cisplatin, 2 cycles) vs no NACT Standard vs high-dose boost for responders	Phase I 45 Gy/25#/33 d 3-wk gap then 15-Gy boost standard arms; 20 Gy–25 Gy boost (high-dose arms) for responders; 40% received brachytherapy boost	NACT with 5-FU 800 mg/m ² days 1–4, 29–32 and cisplatin 80 g/m ² on days 1 and 29; then CRT 5- FU/cisplatin on days as mentioned earlier with RT	Dose escalation of RT boost 15-Gy boost standard arms 20-Gy–25-Gy boost (high-dose arms) for responders	CFS Secondary end points: local control, OS, and cancer-specific survival
ACT II (UKCCCR) (2001–2008) (James et al, ¹¹ 2013)	940	2 × 2 factorial 5-FU/ MMC vs 5-FU cisplatin CRT and consolidation 5-FU/ cisplatin vs control	Phase I 30/6 Gy/17# in 3.5 wk then phase II 19.8 Gy/11# conformal Total 50.4 Gy/28#/38 d <i>no gap</i>	Cisplatin 60 mg/m ² d 1 and 29 vs 12 mg/m ² MMC day 1 with 5-FU 1000 mg/m ² days 1–4, 29–32 CRT	Consolidation 2 courses 5-FU 1000 mg/m ² and cisplatin 60 mg/m ²	Relapse-free survival

Abbreviations: Cisplatin, cisplatinum; HDRT, high-dose radiotherapy; NS, not significant; OTT, overall treatment time; RT, radiotherapy.

allows the cancer cells to repopulate toward the end of radiotherapy, which in theory partly accounts for treatment failure.^{18,19} The only trial that obeyed these constraints and mandated a continuous schedule and prohibited a planned interval was the ACT II trial.¹¹

In this article, the authors discuss the existing evidence basis for the current treatment regimens. The authors review target delineation, optimized radiotherapy techniques, dose-escalation with external-beam radiotherapy (EBRT) or brachytherapy, and chemotherapy. The authors also speculate on novel approaches in terms of the integration of biological agents and immunotherapy, which are being developed for SCCA.

STRATEGIES FOR TREATING LOCALIZED ANAL CANAL CANCER

Radical Surgery

Up until the mid-1980s, the standard treatment of SCCA involved surgery with a permanent colostomy. Local failure rates were reported between 27% and 47% and 5-year OS rates between 40% and 70% (depending on stage and extent of the disease at presentation). Although there has never been a randomized trial comparing radical surgery with radiotherapy or CRT, CRT achieves good local control without the inevitability of a permanent stoma.

Neoadjuvant/Induction Chemotherapy

NACT is widely used in cancers of the head and neck (SCC of the head and neck) before surgery or definitive CRT. In Europe a small pilot study treated SCCA with neoadjuvant 5-FU and cisplatin and showed an 82% response rate, with 27% achieving a complete clinical response (CCR).²⁰ A further study integrated neoadjuvant 5-FU/cisplatin²¹ and showed CCR and partial response (PR) rates of 10% and 51% after NACT, 67% and 28% after CRT, and 93% and 5%, respectively, after all treatment was completed.

In the United States, the Cancer and Leukemia Group B (CALGB 9281) phase II study, for patients with larger, more advanced SCCA (cT3/T4) or with lymph node involvement (cN2/N3), evaluated induction chemotherapy using 5-FU (1000 mg/m² days 1–4 and 29–32) and cisplatin (100 mg/m² on days 1 and 29)²² before CRT. The results showed 8 patients (18%) with CCR and 21 with PR in the 44 evaluable patients, and only a single patient had disease progression. After 4 years of follow-up, 27 of 44 (61%) were disease free.

Two randomized studies (RTOG 9811 and ACCORD-03) examined NACT based on the success of these results. Both incorporated split-course CRT. Additionally RTOG 9811 used 5-FU/cisplatin as the radio-sensitization for the CRT part of the treatment in place of MMC.^{12,17} The results were virtually identical for the CALGB 9281 trial and the RTOG 9811 trial; but in the RTOG 9811 trial, only 26% of patients had tumors greater than 5 cm and positive nodes. Neither of the randomized trials documented the clinical response rates to NACT and whether responding patients had better locoregional control, disease-free survival, and OS.

It remains unclear from the reports of the RTOG 9811 trial whether total dose and/or planning target volumes were modified if patients achieved CCR or near CCR with NACT. There are no specific instructions in protocol. Hence, this may have introduced bias in the different arms, as the authors do not know whether the total dose of radiotherapy differed between induction and the MMC control arm. The studies also fail to report whether the gross target volume and clinical target volume were substantially different between arms or whether the pattern of relapse in the induction arm was different to the MMC control arm.

RADIOTHERAPY

External Beam Radiation Alone

EBRT was originally used as a single modality without chemotherapy. A control rate at the primary site of 75%²³ and OS rates at 5 years of 61% were reported. In a series of 270 patients,²⁴ local control was 90% for tumors less than 4 cm at 10 years but only 65% at 10 years for tumors greater than 4 cm. An early pooled analysis did not show a benefit for 5-FU/MMC in T1 tumors.²⁵ Some units, therefore, continue to treat small T1 tumors with radiation alone, although the ACT I trial suggested CRT is more effective, even for T1 tumors,²⁶ with a hazard ratio in favor of CRT similar to T2 and T3 cancers.

Retrospective studies suggest most recurrences occur locally at the primary site^{1,2,6} often with involvement of adjacent structures and within the high-dose area.¹ Others have reported higher rates of regional recurrence when lower elective nodal doses are used, that is, inguinal failure (40%), common iliac (20%), external iliac (10%), internal iliac (10%), and presacral region (15%).² Nigro⁶ reported that tumors greater than 4 cm in size were more difficult to control. Patients with lesions less than 3 cm in diameter and those treated strictly according to the protocol did significantly better than those with larger lesions and those whose treatment did not comply with the protocol.²⁷

The optimal radiotherapy dose and fractionation for each clinical stage remains unknown. The data from the trials are muddled by limited information on the actual doses received, the overall treatment time (OTT), and the pattern of failure (local, locoregional, or distant). There are no data capturing the sites of locoregional failure in terms of the field size and target delineation, that is, within, marginal to, or outside of the prescribed dose for that radiotherapy field.

Preliminary data from the ACT II trial also suggest failure is usually in-field⁴ and that locoregional failure in the elective nodal volume treated with 30.6 Gy was unusual, suggesting insufficient radiation dose or intrinsic radioresistance rather than inadequate clinical target volumes. Recently, a prospective phase II study evaluating intensity-modulated radiotherapy (IMRT) (RTOG 0529)²⁸ suggested a reduction in acute toxicity compared with the RTOG 9811 trial using 2-dimensional and 3-dimensional techniques. IMRT may reduce the need for treatment breaks, which impact adversely on local control and allow dose-escalation in patients at high risk of local recurrence (T4N0 and T3-4N+). A national IMRT protocol for anal cancer has been developed in the UK (<http://www.analimrtguidance.co.uk/analimrtguidance.pdf>).

RADIOTHERAPY DOSE

In general, larger, more advanced cancers are more difficult to control than smaller ones and require higher doses of radiation, which in turn causes more morbidity. Identification of the optimal radiotherapy dose either with RT alone or CRT has been a challenge. Nigro⁵ originally used a low dose of radiation, 30 Gy, in the CRT schedule as a preoperative neo-adjuvant treatment before surgical resection. RTOG 8314²⁷ delivered 40.08 Gy in 4.5 to 5.0 weeks to the pelvis and perineum. Other prospective series increased the dose to 40 to 45 Gy delivered in 1.8 to 2.0 Gy per fraction²⁹ and achieved a complete response rate of 86%. Studies in older frail adults have also used lower doses for small tumors.^{30,31}

Traditionally, a boost to the tumor with an interval after 45 Gy EBRT with or without chemotherapy has been used to achieve local control in high-risk patients. Most early trials used this schedule.⁷⁻⁹ The ACCORD-03 trial, randomized eligible patients between standard (15 Gy) and higher boost doses (20–25 Gy), but also allowed the use of a higher radiotherapy boost dose if a poor clinical response was observed. The protocol allowed a 3-week gap between CRT and the boost, which will have diluted the effect of the higher dose. In addition, some trials introduced bias by allowing or even

encouraging the investigator to boost with higher total doses if a lack of response in the primary was observed. The RTOG 9811 trial seems to recommend a minimum total dose of 45 Gy for good responders in T1/T2 cancers and higher doses for more advanced tumors or poor responders. The ACT II trial is the only trial that mandated a standard dose of 50.4 Gy to the primary tumor for all stages over 28 daily fractions using a 2-phase technique irrespective of response, with no gap between the phases.¹¹

RTOG 9208 assessed an escalation of the radiotherapy dose to 59.6 Gy over 8.5 weeks with a mandatory 2-week gap. This schedule did not seem to improve local control but showed an increased 1- to 2-year colostomy rate compared with the RTOG 8704 outcomes whereby patients received a dose of only 45.0 to 50.4 Gy in 1.8 Gy per fraction.³² A further 20 patient cohort was recruited with the same dose without treatment breaks, also showed no statistical significant difference in local relapse rate.³³

Higher radiation doses using IMRT are proposed to improve these results, although there are concerns that the function of the sphincter mechanism will be compromised with greater than 54 to 56 Gy leading to fecal incontinence, poor function, and the eventual need for a defunctioning colostomy.³⁴ Long-term fecal incontinence in prostate cancer seems to correlate with the mean dose to the anal-sphincter region, with a cutoff when doses of greater than 40 Gy confer more risk.³⁵ Recent studies suggest that poor sphincter control correlates with doses of 53 Gy or greater received by the lateral extent of the anal sphincter region.³⁶

Late complications of treatment represented 14.5% of the reasons for having a colostomy in the ACCORD-03 trial, that is, 9 of 307 (3%) of the overall population.¹² The RTOG 9811 trial reported severe long-term toxic effects in both arms of 11% and 10%, and 5% of patients required a colostomy for late effects.¹⁰ In ACT II, 14 of 112 (12.5%) patients requiring a posttreatment colostomy needed this because of morbidity, that is, 14 of 940 (1.5%) overall.³⁷

In general, adverse late effects reflect the total radiation dose rather than the type or dose of chemotherapy. With the exception of ACT II, total radiation doses have not been clearly documented in reports of the randomized trials. Sadly ACT II has not reported late toxicity, so a dose response model is, therefore, difficult to calculate.

ELECTIVE DOSE

The appropriate dose to elective nodal areas is also controversial. RTOG 8704 and RTOG 9811 prescribed doses of 30.6 Gy, 36 Gy, and 45 Gy to different areas of clinically negative nodes depending on their anatomic location. However, both the initial United Kingdom Coordinating Committee on Cancer Research (UKCCCR) and European Organization for Research and Treatment of Cancer (EORTC) trials used doses of 45 Gy to elective nodal stations. The RTOG 0529 prescribed 42 to 45 Gy to elective areas that appeared uninvolved on imaging and 50.4 Gy to inguino-pelvic lymph nodes less than 3 cm in size. Lymph nodes larger than 3 cm in size were treated to the same dose as the primary tumor, that is, 54 Gy. The elective nodal dose in ACT II was only 30.6 Gy in 17 daily fractions with large parallel, opposed fields.¹¹ Others have documented that CRT to elective nodal volumes (inguinal area and whole pelvis) over 4 weeks using a dose of 36 Gy achieved excellent nodal control.³⁸ Hence, a total of 30 Gy may be sufficient to treat microscopic nodal deposits and small-volume (<2 cm) macroscopic disease.

BRACHYTHERAPY

Brachytherapy is a highly conformal treatment that can deliver a high dose to the primary tumor, sparing surrounding normal tissues and the contralateral mucosa and

sphincter. Implantation requires local or general anesthesia and demands skill and experience from the operator. Brachytherapy as a single modality is not recommended but may prove useful as a high-dose boost following response to CRT. Often a dose of 45 to 50 Gy using CRT is followed by a 15- to 20-Gy boost, delivered using interstitial brachytherapy. To avoid the risk of necrosis and/or treatment failure, the technique is probably best restricted to tumors that extend less than 50% of the circumference of the anal canal, are less than 5 to 10 mm in thickness, and are less than 5 cm in length.³⁹ Brachytherapy is a potentially useful technique both for the definitive and recurrent/salvage settings, but there is no consensus on the optimal fractionation schedule.⁴⁰ Double-plane or volume implants may be necessary, depending on the extent of the tumor. There is a risk of late necrosis and ongoing radiation proctitis. Recent reports of multiparametric MRI/computed tomography (CT) image adapted brachytherapy show the technique allows excellent dose distribution, but the expertise is limited.⁴¹

MAINTENANCE/CONSOLIDATION CHEMOTHERAPY FOLLOWING CHEMORADIATION

In SCCA a small retrospective study of advanced SCCA (61% were node positive and 43% T3/T4) suggested benefit for consolidation chemotherapy using 8 courses of chemotherapy with 5-FU/cisplatin following definitive CRT.⁴² The ACT II trial in the United Kingdom randomly allocated 930 patients to 2 courses of maintenance/consolidation chemotherapy following CRT (5-FU 750 mg/m² for 4 days and cisplatin 60 mg/m² on day 1) versus control. This additional chemotherapy did not increase the CCR rate, the speed with which the tumor responded, or any long-term oncological outcomes, such as PFS or OS.^{11,43}

THE OVERALL TREATMENT TIME

The interval between the different phases of radiation used in many trials may dilute the biological effects of a small boost of 5 to 10 Gy. Taking into account a repopulation constant with a presumed value of approximately 0.6 Gy/day⁴⁴ means only an additional 2.4 Gy may have been added by the boost in the ACT I trial, although with a 25-Gy boost this would have been closer to 12 Gy.⁷ From these data and considering that local relapse remains the main site of failure, especially in locally advanced disease, a dose escalation might still be feasible and reasonable if IMRT is used and no planned gap is allowed, thus, optimizing the CRT component.

FIELD SIZE

Early trials used generous field sizes applied with parallel opposed beams to avoid a geometric miss, using the pelvic bones as reference points for the position of the pelvic nodes, based on data from lymphangiograms, CT scans and the histopathologic data from early radical surgical series.

Variations in field size exist between studies, particularly in the superior extent. Inclusion of the common iliac nodes, and full coverage of the entire internal iliac nodal system, by setting the upper CTV border at or above the sacral promontory^{1,2} remains controversial. In ACT II the superior aspect of the initial anterior-posterior/posterior-anterior field was defined as 2 cm above the inferior aspect of the sacroiliac (SI) joints, usually at the S1/S2 interface; with beam divergence, the estimated dose to the common iliac nodes was small.⁴⁵ Very few isolated recurrences are observed above this field in the ACT II dataset.⁴ In the RTOG's recommendations, common iliac nodes up to the fifth lumbar vertebra were included in the elective nodal volume to a dose of 45 Gy.^{46,47} In the Norwegian National Population Cohort,⁷ the recommended

superior border was at the level of the lower border of the SI joints. Only if the primary cancer extended into rectal mucosa or the pelvic nodes were considered involved on imaging did the field extend up to the sacral promontory. Despite the fact that approximately 50% of patients had a field only extending to the lower end of the SI joint, no recurrences were observed above this treatment field. Hence, inclusion routinely of common iliac lymph nodes within the radiotherapy field may not be justified.

TARGET DELINEATION

CT planning relies on the contrast in tissue density between different organs. In SCCA, structures around the anal canal blend with indistinct organ borders. MRI can be coregistered to CT scans, offering better spatial resolution than either modality alone and defining tumor size, local extent and spread, invasion of adjacent organs, and nodal involvement.⁴⁸ Most anal carcinomas are also [18F] fluorodeoxyglucose avid, so metabolically active sites in both the primary tumor and normal-sized regional nodes can be identified with superior sensitivity compared with CT alone; but unlike MRI, images are not sharply delineated.

The RTOG, European Society for Radiotherapy and Oncology, and Australasian Gastro-Intestinal Trials Group have produced atlases for target delineation and prophylactic nodal irradiation^{46,49} and also pelvic normal tissue contouring guidelines.⁵⁰ A recent UK initiative has also developed IMRT anal cancer guidance.⁵¹

RATIONALE FOR CHEMORADIOTHERAPY

The randomized trials in SCCA have only examined 3 cytotoxic agents as partners of radiotherapy (5-FU, MMC, and cisplatin). Although the schedules are similar (often days 1–5 and days 29–33), the doses, regimens, and OTT have not been consistent (**Table 2**). The strategy of continuous radio-sensitization throughout the radiotherapy currently accepted in rectal cancer (ie, prolonged venous infusion or oral capecitabine) has infrequently been exploited in anal cancer.^{52,53}

Trials have never directly compared 1 or 2 doses of MMC, and so it remains unclear whether a second dose of MMC as used in some randomized trials^{9,10} adds to efficacy. In Europe, several studies integrated concurrent cisplatin.^{21,54,55} Various cisplatin doses have been explored in these different studies of CRT,^{54,56,57} between 60 and 80 mg/m² on days 1 and 29, without an obvious advantage to the higher dose. An Eastern Cooperative Oncology Group (ECOG) phase II study (E4292) combined 2 courses of cisplatin 75 mg/m² on day 1 and 43 with a high dose of concurrent radiotherapy (59.4 Gy in 33 fractions), with a 2-week break after 36 Gy.⁵⁸ In total, 37% of patients experienced grade 4/5 toxicity. A second cohort received the same treatment without the planned treatment break and showed even more grade 4 toxicities.⁵⁹

Cisplatin has only been explored in a weekly schedule in a single phase II study combined with MMC rather than 5-FU.⁵³ The EORTC 22011-40014 was initially planned as a randomized phase II/III trial designed to assess the response rate in the phase II component. The study compared 5-FU and MMC in combination with radiation versus MMC and cisplatin concurrent with radiation. The response rate of the MMC/cisplatin arm was 91.9%, satisfying the condition for phase III testing; but the investigators thought this regimen was not deliverable in a wider setting. Treatment with MMC has a lower median toxic dose (TD₅₀) and a more steeply increasing normal tissue complication probability curve compared with treatment with cisplatin.⁶⁰

A combination of all 3 drugs in CRT (ie, 5-FU, MMC, and cisplatin) was investigated in a pilot study in the United Kingdom with a regimen using MMC at 10 mg/m² on day 1

Table 2
Outcomes of the more recent (1998–2008) phase III randomized trials in squamous cell carcinoma of the anus, the different chemoradiation regimens and outcomes

Studies (Authors)	Number of Patients	Median Follow-up	CCR	Local Failure Rate	DFS/RFS	Colostomy Rate/CFS	OS
RTOG 9811 (Ajani et al, ¹⁰ 2008)	644	2.5 y	No data on clinical response provided	25% with 5-FU, MMC vs 33% with 5-FU, cisplatin	DFS 60% with 5-FU, MMC vs 54% 5-FU, cisplatin at 5 y; NS $P = .17$	Colostomy rate: 10% with 5-FU, MMC vs 19% with 5-FU, cisplatin; $P = .02$ at 5 y	75% with 5-FU, MMC vs 70% with 5-FU, cisplatin; $P = .1$ at 5 y
ACCORD-03 (Peiffert et al, ¹² 2012)	307	50 mo	Overall 79% complete clinical response at 2 mo after boost	Arm A 28% Arm B 12% Arm C 16% Arm D 22% Overall 19% at 5 y	Tumor-free survival Arm A 64% Arm B 78% Arm C 67% Arm D 62%	5-y CFS Arm A 70% Arm B 82% Arm C 77% Arm D 73%	5-y specific survival Arm A 77% Arm B 89% Arm C 81% Arm D 76%
UKCCCR ACT II (James et al, ¹¹ 2013)	940	36 mo	94.5% 5-FU/MMC/RT vs 95% 5-FU/Cis/RT at 18 wk (ie, 12 wk before)	11% with MMC, 13% with cisplatin	RFS 75% in both arms at 3 y	Colostomy rate: same in both arms (5% with maintenance vs 4% without)	85% with maintenance at 3 y 84% without, NS

Abbreviations: Cis, Cisplatinum; HDRT, high-dose radiotherapy; NS, not significant; RFS, relapse-free survival; RT, radiotherapy.

and cisplatin 60 mg/m² on days 1 and 29,⁶¹ but it was not taken forward because of concerns regarding acute toxicity and poor compliance.

ORAL FLUOROPYRIMIDINES

Capecitabine was successfully substituted for infusional 5-FU in a phase II study using 825 mg/m² twice a day on radiation days and MMC on day 1.⁶² A more recent phase I study confirmed that 825 mg/m² twice a day orally is tolerable and seems effective.⁶³ Other phase II studies⁶⁴ and retrospective series^{65,66} have confirmed this dosage as tolerable and effective (Table 3).

OTHER CYTOTOXIC AGENTS

A recent phase I trial of IMRT and concurrent chemotherapy using paclitaxel, capecitabine, and MMC for SCCA is reported in abstract. The regimen seems feasible in terms of toxicity, and 18 of 20 patients (90%) had a complete response at 6 months.⁶⁷ Based on these results, a phase III clinical trial is planned comparing the triplet regimen (paclitaxel, capecitabine, and MMC) combined with IMRT against the standard doublet of capecitabine and MMC alone (NCT02526953).

EPIDERMAL GROWTH FACTOR RECEPTOR INHIBITION

Epidermal growth factor receptor (EGFR) is overexpressed in up to 90% of SCCA. EGFR mutations, Kirsten rat sarcoma viral oncogene mutations, or BRAF mutations have rarely been identified in SCCA,^{68,69} although one study showed PIK3CA was found to be mutated in 22%.⁶⁹ Thus, EGFR inhibition is a potential strategy to integrate

Table 3 Showing results of phase I/phase II and retrospective studies integrating capecitabine into chemoradiation regimens					
Study	Number of Patients	Radiotherapy Dose	Schedule of MMC	Capecitabine	Local Control
Glynne-Jones et al, ⁶² 2008	31	50.4 Gy in 28 fractions in 2 phases	Single dose of MMC 12 mg/m ² max 20 mg	825 mg/m ² bid on radiation days	90% at 14 m
Deenen et al, ⁶³ 2013	18	59.4 Gy in 33 fractions with simultaneous integrated boost-IMRT	Single dose of MMC 10 mg/m ² max 15 mg	825 mg/m ² bid on radiation days	88% at 28 m
Oliveira et al, ⁶⁴ 2016	51	Not stated	Single dose of MMC 15 mg/m ² on day 1	825 mg/m ² bid on radiation days	86% at 6 m
Thind et al, ⁶⁵ 2014	66	Median dose of radiation 51.9 Gy over 5.5 wk	Single dose of MMC 12 mg/m ²	825 mg/m ² bid on radiation days	94% at 20 m
Wan et al, ⁶⁶ 2014	300	50–54 Gy	Two doses But compliance poor if capecitabine	825 mg/m ² bid on radiation days	DFS (at 1 y) 94% cape vs 91% 5-FU

into concurrent CRT treatment. In SCCA, several phase I studies investigated the addition of cetuximab to 5-FU and cisplatin-based CRT.⁷⁰ A response rate of 95% was observed, but the study closed early because of toxicity (52% showing severe grade 3–4 radiation dermatitis and 44% experiencing grade 3–4 diarrhea). The ACCORD 16 phase I trial also closed early because of unacceptable toxicity⁷¹ and poor oncological outcomes.⁷² Other phase II studies (ECOG 3205) for immunocompetent patients and (AIDS Malignancy Consortium Trial [AMC045]) human immunodeficiency virus (HIV)-positive patients showed acceptable toxicity and clinical outcomes for patients treated with the combination of cetuximab with 5-FU and cisplatin concurrently with radiation.⁷³ Other studies have combined cetuximab with 5-FU/MMC-based CRT.⁷⁵ Toxicity was acceptable, and 10 of 11 patients (91%) achieved a local complete remission (CR). A phase 2 trial with panitumumab, mitomycin-C, 5-FU, and radiotherapy in SCCA, has been developed by the Grupo Espanol Multidisciplinar en Cancer Digestivo (GEMCAD) - (NCT01285778).^{76,77} Preliminary results showed only 55% had CR at 24 weeks ([Table 4](#)).

IMMUNOTHERAPY

Strong data support HPV infection as an early event in SCCA with reports of 84% to 85% HPV prevalence.^{78,79} The two most common genotypes are HPV 16 and HPV 18. It is postulated that HPV integration at an early stage and loss of heterozygosity at multiple chromosomal sites may lead to anal carcinogenesis. Therefore, immunotherapy seems a promising approach and, as such, is being studied in combination with CRT.

Advaxis (ADXS) 11-001 is a bioengineered *Listeria monocytogenes*-based treatment combining live attenuated *Listeria* bacteria fused to HPV16E7 causing a cell-mediated immune response. ADXS 11-001 is being evaluated in a phase I/II study of 25 patients in combination with 5-FU and MMC and IMRT for anal cancer; this trial is currently recruiting (NCT01671488). ADXS 11-001 is also being tested with CRT in several other HPV-positive cancers, such as head and neck and cervical cancer.

The inhibitory molecules programmed cell death 1 (PD-1) and its ligands, PD-L1 and PD-L2, are often upregulated and lead to suppression of the antitumor immune response in many cancers. PDL1 expression has been associated with a worse prognosis; hence, checkpoint inhibition has proved successful in other tumor types. There is a paucity of data in SCCA, but one study of 41 patients evaluated PDL1 expression retrospectively using paraffin tissue blocks and identified 62% PDL1-positive in the late stage and 33% positive in the early stage. Although there were very small numbers, there was a suggestion of poorer relapse-free survival; but OS was not different between PDL1 positive and negative groups. Trials combining CRT with PDL1 blockade for anal cancer are currently being planned but will require cautious overview because of the potential overlapping gastrointestinal toxicities with CRT.

An evaluation of tumor-infiltrating lymphocytes (TIL) scores after chemoradiotherapy provides further support for immunotherapy in this setting.⁸⁰ P16 immunohistochemistry has been used as a surrogate for HPV involvement and demonstrated poor overall outcomes for HPV/p16-negative patients in several series.^{81,82} One plausible explanation has been that the papillomavirus evokes a host immune response directed against tumor cells. In oropharyngeal cancer HPV 16–E6 and E7-specific T cells have been found and correlate with improved outcome. Thus, Gilbert and colleagues⁸⁰ assessed TIL to stratify p16-positive cases and demonstrated additional prognostic value with this approach. TILs remained an independent prognostic score in a multivariate analysis with relapse-free rates of 92% with high levels of TIL versus 63% in absent/low-level TIL.⁸⁰ These data support the use of immunotherapy either

Table 4**Showing preliminary and full results of phase I/phase II studies integrating cetuximab or panitumumab into chemoradiation regimens**

Trial	No of Patients	IMRT	Regimen	Toxicity	Efficacy
Phase I Olivatto et al, ⁷⁰ Cancer 2013	21 (stopped because of DLT)	No	5-FU/CP + RT + cetuximab	High	OK
Phase I ACCORD 16 Deutsch et al, ⁷¹ Ann Oncol 2013 Levy et al, ⁷² Radiother Oncol 2015	16 (stopped because of DLT)	No	5-FU/CP + RT + cetuximab	High	Low
Phase I Garg et al, ⁷³ ECOG 3205, J Clin Oncol 2012 Garg et al, ⁷⁴ J Clin Oncol 2016	28	Some	5-FU/CP + RT + cetuximab	G4 toxicity 32% G5 4%	93% OS at 2 y
Phase I Garg et al, ⁷⁴ AMC045, J Clin Oncol 2016	45	Some	5-FU/CP + RT + cetuximab	G4 toxicity 26% G5 4%	89% OS at 2 y
Phase I Leon et al, ⁷⁵ 2015	13	Yes	5-FU/MMC + RT + cetuximab	Low	73% CR at 3 mo
Phase II Feliu, ⁷⁷ ASCO 2014	58 36 evaluable	No	5-FU/MMC + RT + panitumumab	High	At 24 wk 55% CR, 6% persistent disease; 19% progression

with concurrent CRT or using adoptive T-cell therapy that is being studied in trials in the advanced SCCA setting.

The Sonic hedgehog (SHH) glycoprotein is part of a complex signaling cascade that is regulated by Gli1. Ajani and colleagues⁸³ demonstrated that overexpression of Gli 1 and SHH glycoprotein were potentially predictive for reduced DFS.^{83,84} These data support further interrogation of this pathway, especially as SHH signaling has been correlated with resistance to CRT in other cancers.

SUMMARY

Current guidelines from the European Society for Medical Oncology and the National Comprehensive Cancer Network recommend the use of concurrent CRT with 5-FU and MMC for radical treatment of local and locally advanced SCCA. Yet, we have not confirmed the optimal dose of radiotherapy or defined the schedules with the optimal sequence and timing of the available cytotoxic agents (5-FU, MMC, and cisplatin). Oral capecitabine offers an alternative to 5-FU but has not been directly compared with intravenous 5-FU in trials. Studies using the targeted agent cetuximab seem to cause unexpected toxicity, without showing any evidence of increased efficacy.

The response to chemoradiation is heterogeneous and may reflect the tumor environment and the immunologic capacity as much as innate radio-sensitivity. Biomarkers in development (p16, squamous cell carcinoma antigen, PET/CT, Tumor infiltrating lymphocytes, PD-1 expression, SHH expression) may stratify SCCA more accurately and guide the individual intensity of treatment required. In the future, we should integrate new agents, such as taxanes, checkpoint inhibitors, and PD-1, and molecularly targeted agents into CRT. The greater precision of IMRT can spare critical normal tissues, thereby reducing acute toxicity and potentially allowing the safe use of dose escalation. We also need to design trials specifically for patients with HPV-negative and HPV-positive SCCA both in smokers and nonsmokers, respectively, according to different TNM staging.

REFERENCES

1. Das P, Bhatia S, Eng C, et al. Predictors and patterns of recurrence after definitive chemoradiation for anal cancer. *Int J Radiat Oncol Biol Phys* 2007;68(3):794–800.
2. Wright JL, Patil S, Temple L, et al. Squamous cell carcinoma of the anal canal: patterns and predictors of failure and implications for intensity modulated radiation treatment planning. *Int J Radiat Oncol Biol Phys* 2009;78(4):1064–72.
3. Northover J, Glynne-Jones R, Sebag-Montefiore D, et al. Chemoradiation for the treatment of epidermoid anal cancer: 13-year follow-up of the first randomised UKCCCR Anal Cancer Trial (ACT I). *Br J Cancer* 2010;102(7):1123–8.
4. Sebag-Montefiore D, James R, Meadows H, et al. The pattern and timing of disease recurrence in squamous cancer of the anus: mature results from the NCRI ACT II trial. *J Clin Oncol* 2012;30(Suppl) [abstract: 4029].
5. Nigro ND, Vaitkevicius VK, Considine B Jr. Combined therapy for cancer of the anal canal: a preliminary report. *Dis Colon Rectum* 1974;17(3):354–6.
6. Nigro ND. An evaluation of combined therapy for squamous cell carcinoma of the anal canal. *Dis Colon Rectum* 1984;27:763–6.
7. UKCCCR Anal Cancer Working Party. Epidermoid Anal Cancer: results from the UKCCCR randomised trial of radiotherapy alone versus radiotherapy, 5-fluorouracil and mitomycin. *Lancet* 1996;348:1049–54.
8. Bartelink H, Roelofsen F, Eschwege F, et al. Concomitant radiotherapy and chemotherapy is superior to radiotherapy alone in the treatment of locally

- advanced anal cancer: results of a phase III randomized trial of the European Organization for Research and Treatment of Cancer Radiotherapy and Gastrointestinal Cooperative Groups. *J Clin Oncol* 1997;15(5):2040–9.
9. Flam M, John M, Pajak TF, et al. Role of mitomycin in combination with fluorouracil and radiotherapy, and of salvage chemoradiation in the definitive nonsurgical treatment of epidermoid carcinoma of the anal canal: results of a phase III randomized intergroup study. *J Clin Oncol* 1996;14(9):2527–39.
 10. Ajani JA, Winter KA, Gunderson LL, et al. Fluorouracil, mitomycin, and radiotherapy vs fluorouracil, cisplatin, and radiotherapy for carcinoma of the anal canal: a randomized controlled trial. *JAMA* 2008;299(16):1914–21.
 11. James RD, Glynn-Jones R, Meadows H, et al. 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, 2x2 factorial trial. *Lancet Oncol* 2013;14:516–24.
 12. Peiffert D, Tournier-Rangeard L, Gerard JP, et al. Induction chemotherapy and dose intensification of the radiation boost in locally advanced anal canal carcinoma: final analysis of the randomized UNICANCER ACCORD 03 trial. *J Clin Oncol* 2012;30:1941–4.
 13. Glynn-Jones R, Nilsson PJ, Aschele C, et al. Anal cancer: ESMO-ESSO-ESTRO clinical practice guidelines for diagnosis treatment and follow up. *Ann Oncol* 2014;25(Suppl 3):iii10–20.
 14. NCCN. NCCN Clinical Practice Guidelines in Oncology: anal carcinoma, version 2, 2016. Available at: https://www.nccn.org/professionals/physician_gls/f_guidelines.asp. Accessed August 12, 2016.
 15. Gunderson LL, Winter KA, Ajani JA, et al. Long-term update of U.S. GI Intergroup RTOG 98-11 phase III trial for anal carcinoma: survival, relapse and colostomy failure with concurrent chemoradiation involving fluorouracil/mitomycin versus fluorouracil/cisplatin. *J Clin Oncol* 2012;30:4344–51.
 16. Bentzen AG, Guren MG, Vonen B, et al. Faecal incontinence after chemoradiotherapy in anal cancer survivors: long-term results of a national cohort. *Radiother Oncol* 2013;108(1):55–60.
 17. Gunderson LL, Moughan J, Ajani JA, et al. Anal carcinoma: impact of TN category of disease on survival, disease relapse, and colostomy failure in US Gastrointestinal Intergroup RTOG 98-11 phase 3 trial. *Int J Radiat Oncol Biol Phys* 2013;87(4):638–45.
 18. Withers HR, Taylor JMF, Maciejewski B. The hazard of accelerated tumour clonage and repopulation during radiotherapy. *Acta Oncol* 1988;27:131–46.
 19. Fowler JF, Lindstrom MJ. Loss of local control with prolongation in radiotherapy. *Int J Radiat Oncol Biol Phys* 1992;23:457–67.
 20. Brunet R, Sadek H, Vignoud J, et al. Cisplatin (P) and 5 fluorouracil (5FU), for the neoadjuvant treatment (Tt) of epidermoid anal canal carcinoma (EACC) [abstract]. *Proc Am Soc Clin Oncol* 1990;9:104.
 21. Peiffert D, Giovannini M, Ducreux M, et al. Digestive Tumours Group of the French 'Fédération Nationale des Centres de Lutte Contre le Cancer'. High-dose radiation therapy and neoadjuvant plus concomitant chemotherapy with 5-fluorouracil and cisplatin in patients with locally advanced squamous-cell anal canal cancer: final results of a phase II study. *Ann Oncol* 2001;12(3):397–404.
 22. Meropol NJ, Niedzwiecki D, Shank B, et al. Induction therapy for poor-prognosis anal canal carcinoma: a phase II study of the cancer and leukemia group B (CALGB 9281). *J Clin Oncol* 2008;26(19):3229–34.

23. Ng Ying Kin NY, Pigneux J, Auvray H, et al. Our experience of conservative treatment of anal canal carcinoma combining external irradiation and interstitial implant: 32 cases treated between 1973 and 1982. *Int J Radiat Oncol Biol Phys* 1988;14(2):253–9.
24. Touboul E, Schlienger M, Buffat L, et al. Epidermoid carcinoma of the anal canal. Results of curative-intent radiation therapy in a series of 270 patients. *Cancer* 1994;73(6):1569–79.
25. Cummings BJ, Keane TJ, O'Sullivan B, et al. Epidermoid anal cancer: treatment by radiation alone or by radiation and 5-fluorouracil with and without mitomycin C. *Int J Radiat Oncol Biol Phys* 1991;21(5):1115–25.
26. Northover J, Meadows H, Ryan C, et al. Combined radiotherapy and chemotherapy for anal cancer. *The Lancet* 1997;349:205–6.
27. Sischy B, Doggett RL, Krall JM, et al. Definitive irradiation and chemotherapy for radiosensitization in management of anal carcinoma: interim report on Radiation Therapy Oncology Group Study No. 8314. *J Natl Cancer Inst* 1989;81:850–6.
28. Kachnic LA, Winter K, Myerson RJ, et al. RTOG 0529: a phase 2 evaluation of dose-painted intensity modulated radiation therapy in combination with 5-fluorouracil and mitomycin-C for the reduction of acute morbidity in carcinoma of the anal canal. *Int J Radiat Oncol Biol Phys* 2013;86(1):27–33.
29. Flam MS, John MJ, Mowry PA, et al. Definitive combined modality therapy of carcinoma of the anus. A report of 30 cases including results of salvage therapy in patients with residual disease. *Dis Colon Rectum* 1987;30(7):495–502.
30. Hu K, Minsky BD, Cohen AM, et al. 30 Gy may be an adequate dose in patients with anal cancer treated with excisional biopsy followed by combined-modality therapy. *J Surg Oncol* 1999;70:71–7.
31. Hatfield P, Cooper R, Sebag-Montefiore D. Involved-field, low-dose chemoradiotherapy for early-stage anal carcinoma. *Int J Radiat Oncol Biol Phys* 2008;70:419–24.
32. John M, Pajak T, Flam M, et al. Dose escalation in chemoradiation for anal cancer: preliminary results of RTOG 92-08. *Cancer J Sci Am* 1996;2:205–11.
33. Konski A, Garcia M Jr, John M, et al. Evaluation of planned treatment breaks during radiation therapy for anal cancer: update of RTOG 92-08. *Int J Radiat Oncol Biol Phys* 2008;72:114–8.
34. Broens P, Van Limbergen E, Penninckx F, et al. Clinical and manometric effects of combined external beam irradiation and brachytherapy for anal cancer. *Int J Colorectal Dis* 1998;13:68–72.
35. Alsadius D, Hedelin M, Lundstedt D, et al. Mean absorbed dose to the anal-sphincter region and fecal leakage among irradiated prostate cancer survivors. *Int J Radiat Oncol Biol Phys* 2012;84(2):e181–5.
36. Buettner F, Gulliford SL, Webb S, et al. The dose-response of the anal sphincter region—an analysis of data from the MRC RT01 trial. *Radiother Oncol* 2012;103(3):347–52.
37. Glynne-Jones R, Kadalayil L, Meadows HM, et al, on behalf of ACT II Study Group. Tumour- and treatment-related colostomy rates following mitomycin C or cisplatin chemoradiation with or without maintenance chemotherapy in squamous cell carcinoma of the anus in the ACT II trial. *Ann Oncol* 2014;25(8):1616–22.
38. Lepinoy A, Lescut N, Puyraveau M, et al. Evaluation of a 36Gy elective node irradiation dose in anal cancer. *Radiother Oncol* 2015;116(2):197–201.
39. Mazoner JJ, van Limbergen E. Anorectal cancer. In: Gerbaulet A, Pötter R, Mazoner J, et al, editors. *The GECESTROH and book of brachytherapy*. Brussels (Belgium): ESTRO; 2000. p. 505–14.
40. Niehoff P, Kovacs G. HDR brachytherapy for anal cancer. *J Gastrointest Oncol* 2014;5(3):218–22.

41. Tagliaferri L, Manfrida S, Barbaro B, et al. MITHRA – multiparametric MR/CT image adapted brachytherapy (MR/CT-IABT) in anal canal cancer: a feasibility study. *J Contemp Brachytherapy* 2015;7(5):336–45.
42. Glynne-Jones R, James R, Meadows H, et al. 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. *J Clin Oncol* 2012;30(Suppl) [abstract: 4004].
43. Cho BC, Ahn JB, Seong J, et al. Chemoradiotherapy with or without consolidation chemotherapy using cisplatin and 5-fluorouracil in anal squamous cell carcinoma: long-term results in 31 patients. *BMC Cancer* 2008;15:8.
44. Glynne-Jones R, Sebag-Montefiore D, Adams R, et al. “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(5): 1488–94.
45. Aggarwal A, Gayadeen S, Robinson D, et al. Clinical target volumes in anal cancer: calculating what dose was likely to have been delivered in the UK ACT II trial protocol. *Radiother Oncol* 2012;103(3):341–6.
46. Myerson RJ, Garofalo MC, Naqa I, et al. Elective clinical target volumes for conformal therapy in anorectal cancer: a radiation therapy oncology group consensus panel contouring atlas. *Int J Radiat Oncol Biol Phys* 2009;74(3): 824–30.
47. Bentzen AG, Guren MG, Wanderås EH, et al. Chemoradiotherapy of anal carcinoma: survival and recurrence in an unselected national cohort. *Int J Radiat Oncol Biol Phys* 2012;83(2):e173–80.
48. Goh V, Gollub FK, Liaw J, et al. Magnetic resonance imaging assessment of squamous cell carcinoma of the anal canal before and after chemoradiation: can MRI predict for eventual clinical outcome? *Int J Radiat Oncol Biol Phys* 2010;78(3):715–21.
49. Ng M, Leong T, Chander S, et al. Australasian Gastrointestinal Trials Group (AGITG) contouring atlas and planning guidelines for intensity-modulated radiotherapy in anal cancer. *Int J Radiat Oncol Biol Phys* 2012;83:1455–62.
50. Gay HA, Barthold HJ, O’Meara E, et al. Pelvic normal tissue contouring guidelines for radiation therapy: a Radiation Therapy Oncology Group consensus panel atlas. *Int J Radiat Oncol Biol Phys* 2012;83:e353–62.
51. Muirhead R, Adams RA, Gilbert DC, et al. Anal cancer: developing an intensity-modulated radiotherapy solution for ACT2 fractionation. *Clin Oncol (R Coll Radiol)* 2014;26:720–1.
52. Bosset JF, Roelofsen F, Morgan DA, et al. Shortened irradiation scheme, continuous infusion of 5-fluorouracil and fractionation of mitomycin C in locally advanced anal carcinomas. Results of a phase II study of the European Organization for Research and Treatment of Cancer. Radiotherapy and Gastrointestinal Cooperative Groups. *Eur J Cancer* 2003;39(1):45–51.
53. Matzinger O, Roelofsen F, Mineur L, et al. Mitomycin C with continuous fluorouracil or with cisplatin in combination with radiotherapy for locally advanced anal cancer (European Organisation for Research and Treatment of Cancer phase II study 22011-40014). *Eur J Cancer* 2009;45(16):2782–91.
54. Doci R, Zucali R, Bombelli L, et al. Combined chemoradiation therapy for anal cancer. A report of 56 cases. *Ann Surg* 1992;215(2):150–6.
55. Gerard JP, Ayzac L, Hun D, et al. Treatment of anal canal carcinoma with high dose radiation therapy and concomitant fluorouracil-cisplatin. Long-term results in 95 patients. *Radiother Oncol* 1998;46(3):249–56.

56. Hung A, Crane C, Delclos M, et al. Cisplatin-based combined modality therapy for anal carcinoma: a wider therapeutic index. *Cancer* 2003;97(5):1195–202.
57. Olivatto LO, Cabral V, Rosa A, et al. Mitomycin-C- or cisplatin-based chemoradiotherapy for anal canal carcinoma: long-term results. *Int J Radiat Oncol Biol Phys* 2011;79(2):490–5.
58. Martenson JA, Lipsitz SR, Wagner H Jr, et al. Initial results of a phase II trial of high dose radiation therapy, 5-fluorouracil, and cisplatin for patients with anal cancer (E4292): an Eastern Cooperative Oncology Group study. *Int J Radiat Oncol Biol Phys* 1996;35(4):745–9.
59. Chakravarthy AB, Catalano PJ, Martenson JA, et al. Long-term follow-up of a phase II trial of high-dose radiation with concurrent 5-fluorouracil and cisplatin in patients with anal cancer (ECOG E4292). *Int J Radiat Oncol Biol Phys* 2011; 81(4):e607–13.
60. Bazan JG, Luxton G, Kozak MM, et al. Impact of chemotherapy on normal tissue complication probability models of acute hematologic toxicity in patients receiving pelvic intensity modulated radiation therapy. *Int J Radiat Oncol Biol Phys* 2013;87(5):983–91.
61. Sebag-Montefiore D, Meadows HM, Cunningham D, et al. Three cytotoxic drugs combined with pelvic radiation and as maintenance chemotherapy for patients with squamous cell carcinoma of the anus (SCCA): long-term follow-up of a phase II pilot study using 5-fluorouracil, mitomycin C and cisplatin. *Radiother Oncol* 2012;104(2):155–60.
62. Glynne-Jones R, Meadows H, Wan S, et al. EXTRA—a multicenter phase II study of chemoradiation using a 5 day per week oral regimen of capecitabine and intravenous mitomycin C in anal cancer. *Int J Radiat Oncol Biol Phys* 2008;72(1):119–26.
63. Deenen MJ, Dewit L, Boot H, et al. Simultaneous integrated boost-intensity modulated radiation therapy with concomitant capecitabine and mitomycin C for locally advanced anal carcinoma: a phase 1 study. *Int J Radiat Oncol Biol Phys* 2013; 85(5):e201–7.
64. Oliveira SC, Moniz CM, Riechelmann R, et al. Phase II study of capecitabine in substitution of 5-FU in the chemoradiotherapy regimen for patients with localized squamous cell carcinoma of the anal canal. *J Gastrointest Cancer* 2016;47(1): 75–81.
65. Thind G, Johal B, Follwell M, et al. Chemoradiation with capecitabine and mitomycin-C for stage I-III anal squamous cell carcinoma. *Radiat Oncol* 2014; 9:124.
66. Wan DDC, Schellenberg D, Hay J, et al. A comparison between 5-fluorouracil/mitomycin (FM) and capecitabine/mitomycin (CM) in combination with radiation (RT) for squamous cell carcinoma (SCC) of the anal canal. *J Clin Oncol* 2014; 32(5s) [abstract: 4031].
67. Gordeev S, Rasulov A, Gorbunova V, et al. A pilot study of intensity-modulated radiation therapy (IMRT) and concurrent chemotherapy (CT) with paclitaxel, capecitabine and mitomycin C for squamous cell anal carcinoma (SCAC). *J Clin Oncol* 2015;33(Suppl) [abstract: e14501].
68. Casadei Gardini A, Capelli L, Ulivi P, et al. KRAS, BRAF and PIK3CA status in squamous cell anal carcinoma (SCAC). *PLoS One* 2014;9(3):e92071.
69. Serup-Hansen E, Linnemann D, Høgdall E, et al. KRAS and BRAF mutations in anal carcinoma. *APMIS* 2015;123(1):53–9.
70. Olivatto LO, Vieira FM, Pereira BV, et al. Phase 1 study of cetuximab in combination with 5-fluorouracil, cisplatin, and radiotherapy in patients with locally advanced anal canal carcinoma. *Cancer* 2013;119(16):2973–80.

71. Deutsch E, Lemanski C, Pignon JP, et al. 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;24:2834–8.
72. Levy A, Azria D, Pignon JP, et al. Low response rate after cetuximab combined with conventional chemoradiotherapy in patients with locally advanced anal cancer: long-term results of the UNICANCER ACCORD 16 phase II trial. *Radiother Oncol* 2015;114(3):415–6.
73. Garg M, Lee JY, Kachnic LA, et al. Phase II trials of cetuximab (CX) plus cisplatin (CDDP), 5-fluorouracil (5-FU) and radiation (RT) in immunocompetent (ECOG 3205) and HIV-positive (AMC045) patients with squamous cell carcinoma of the anal canal (SCAC): safety and preliminary efficacy results. *J Clin Oncol ASCO Annual Meeting Proceedings*. 2012. [abstract: 4030].
74. Garg M, Lee JY, Lisa A, et al. Phase II trials of cetuximab (CX) plus cisplatin (CDDP), 5-fluorouracil (5-FU) and radiation (RT) in immunocompetent (ECOG 3205) and HIV-positive (AMC045) patients with squamous cell carcinoma of the anal canal (SCAC): Safety and preliminary efficacy results. *J Clin Oncol* 34, 2016 (suppl; abstr 3522).
75. Leon O, Guren MG, Radu C, et al. Phase I study of cetuximab in combination with 5-fluorouracil, mitomycin C and radiotherapy in patients with locally advanced anal cancer. *Eur J Cancer* 2015;51(18):2740–6.
76. Moreno V, García-Carbonero R, Maurel J, et al. Phase 1 study of cetuximab in combination with 5-fluorouracil, cisplatin, and radiotherapy in patients with locally advanced anal canal carcinoma. *Cancer* 2014;120(3):454–6.
77. Feliu J, Garcia-Carbonero R, Capdevilla J, et al. Phase II trial of panitumumab (P) plus mitomycin C (M), 5-fluorouracil (5-FU), and radiation (RT) in patients with squamous cell carcinoma of the anal canal (SCAC): safety and efficacy profile—VITAL study, GEMCAD 09-02 clinical trial. *J Clin Oncol* 2014;32(5s) [abstract: 4034].
78. Hoots BE, Palefsky JM, Pimenta JM, et al. Human papilloma virus type distribution in anal cancer and anal intraepithelial lesions. *Int J Cancer* 2009;124:2375–83.
79. Palmer JG, Scholefield JH, Coates PJ, et al. Anal cancer and human papilloma viruses. *Dis Colon Rectum* 1989;32:16–22.
80. Gilbert DC, Serup-Hansen E, Linnemann D, et al. Tumour infiltrating lymphocytes scores effectively stratify outcomes over and above p16 post chemoradiotherapy in anal cancer. *Br J Cancer* 2016;114:134–7.
81. Koerber SA, Schoneweg C, Slynko A, et al. Influence of human papillomavirus and p16 on treatment outcome of patients with anal cancer. *Radiother Oncol* 2014;113:331–6.
82. Rodel F, Wieland U, Fraunholz I, et al. Human papilloma virus DNA load and p16INK4a expression predict for local control in patients with anal squamous carcinoma treated with chemoradiotherapy. *Int J Cancer* 2014;136:278–88.
83. Ajani JA, Wang X, Izzo JG, et al. Molecular biomarkers correlate with disease free survival in patients with anal cell carcinoma treated with chemoradiation. *Dig Dis Sci* 2010;55:1098–105.
84. Sims-Mourtada J, Izzo JG, Apisarnthanarax S, et al. Hedgehog: an attribute to tumour regrowth after chemoradiotherapy and a target to improve radiation response. *Clin Cancer Res* 2006;12:6565–72.