Chapter

Evolving Concepts toward Individualized Treatment of Squamous Cell Carcinoma of the Anus

Luc Dewit, Annemieke Cats and Geerard Beets

"If you do not change direction, you may end up where you are heading",
Lao Tsu, Chinese philosopher.

Abstract

Treatment of squamous cell carcinoma of the anus has evolved over the last 5 decades from radical surgery to combined chemoradiation therapy. Radiation treatment techniques have dramatically improved with the development of more powerful computers, algorithms and treatment machines. The clinical impact of the modern radiation treatment techniques, such as intensity-modulated radiotherapy and volumetric modulated arc therapy, is discussed. The standard-of-care regimen still is concurrent Mitomycin C, 5-fluorouracil and high-dose radiation, as was conceived 45 years ago. Variants of this schedule are discussed in this chapter. International guidelines have been generated and implemented. Whereas concurrent chemoradiation therapy is the treatment of choice for locally advanced tumors, early tumors are probably adequately controlled with either reduced dose chemoradiation therapy or radiation therapy alone. Prognostic factors, such as high-risk human papillomavirus, epidermal growth factor receptor and immune response, will be highlighted. The role of surgery in primary care is limited to local excision of T1N0 tumors ≤ 1 cm of the anal margin. Salvage radical surgery is limited to locoregional recurrent, non-metastasized and resectable tumors after chemoradiation therapy. In addition, new treatment modalities, such as targeted therapy and immunotherapy, will be discussed. Current research aims at refining prognostic subgroups to further individualize treatment strategy, implementing quality assurance protocols in international trials and investigating the molecular profile of squamous cell carcinoma of the anus, in order to identify new treatment avenues. This will hopefully change the landscape of anal cancer treatment in the future.

Keywords: anal carcinoma, radiotherapy, chemoradiation therapy, prognostic factors, surgery, biological agents

1. Introduction

Squamous cell carcinoma of the anus (SCCA) is a rare tumor with an increasing incidence over the last decades [1]. It originates from the basal cells of the epithelial

layer of the anal canal, which extends from the anorectal junction to the anal orifice, or anal margin, which extends from the anal orifice to a radius of 5 cm laterally [2]. Tumors arising from the anal margin have a different biological behavior, and this will be briefly discussed later in this chapter. Most, but not all, SCCA are causally related with high-risk human papillomavirus (HPV-HR), mainly subtypes 16 and 18 [3, 4]. These tumors develop from high grade anal intraepithelial neoplasia (AIN3) through a number of consecutive oncogenic steps, which are only partially understood [5]. Radical surgery, which usually implies an abdominoperineal resection with a permanent end colostomy, has been shown to yield 5-year survival rates of only 20–70%, depending on stage and resection margins [6]. Radiation therapy has demonstrated superior survival rates with a high probability of organ preservation. The seminal papers of Nigro and colleagues have shown that the combination of radiation and chemotherapy resulted in even better survival rates, at least for locally advanced cases [7, 8]. This has been confirmed in two landmark randomized phase III trials [9, 10]. Hence, chemoradiation therapy (CRT) has largely replaced radical surgery in the treatment of SCCA.

The focus of this chapter is to highlight the evolving concepts toward individualized treatment of patients with SCCA, based upon prognostic parameters. Emphasis will be given to improved radiation treatment techniques, concurrent and (neo) adjuvant chemotherapy regimens, the role of HPV status, molecular markers and immune response. In addition, the role of surgery will be addressed.

2. Improved treatment of SCCA

2.1 Technical improvement of radiation treatment of SCCA

2.1.1 Radiation dose and target volume

The efficacy of (chemo)radiation treatment for SCCA has been known for several decades. The acute and late toxicity, however, was considerable with the large, non-conformal treatment fields, which often resulted in moderate functional outcome and quality of life [11]. With the development of more powerful computers, algorithms and treatment machines, more sophisticated treatment techniques became available. This has resulted in a shift from standard opposed anterior-posterior fields (AP-PA) or a four-field technique in the fifties through eighties of the previous century to 3D-conformal radiotherapy (3D-CRT) in the nineties and intensity-modulated radiotherapy (IMRT) in the early years of this century and volumetric modulated arc therapy (VMAT) in the last decade.

The difference in toxicity between 3D-CRT and IMRT or VMAT has never been compared in a prospective randomized trial, but several retrospective studies and one recent prospective study have reported an improved toxicity profile with the newer techniques [12–17]. A recent national audit in the UK comparing these techniques confirmed the reduced toxicity with IMRT (**Table 1**) [18]. A few studies also claim a better disease-free survival (DFS) and locoregional control (LRC) with IMRT [12, 14, 19].

Toxicity is largely related to the radiation dose and the volume of normal tissues exposed to radiation, which in turn is related to the gross tumor volume (GTV) and clinical and planning target volume (CTV and PTV). The GTV is determined by the macroscopic local tumor extent and documented macroscopically involved regional lymph nodes, whereas the CTV is dependent on the site of regional lymph nodes that are considered to be at risk for microscopic metastatic disease. In addition, the PTV is determined by the set-up error of patient positioning. With the advent of magnetic resonance imaging (MRI) and fluor-18-deoxyglucose positron

Comparison of grade 3+4 acute toxicity during chemoradiotherapy (CRT) seen in the ACT2 publication, all UK audit patients, UK audit patients undergoing ACT2 regimen and UK audit patient treated in keeping with UK intensity-modulated radiotherapy (IMRT) guidance

	ACT2 trial* ($n = 472$†)	All UK audit patients ($n = 199$ non-haematological; $n = 192$ haematological*‡)	Two-phase conformal CRT in UK audit ($n = 45$*‡)	IMRT as per guidance in UK audit ($n = 127$ non-haematological; $n = 120$ haematological*‡)
Non-haematological§	294 (62%)	87 (44%)	22 (49%)	51 (40%)
Gastrointestinal	75 (16%)	26 (13%)	5 (11%)	17 (13%)
Nausea	10 (2%)	6 (3%)	2 (4%)	4 (3%)
Vomiting	9 (2%)	4 (2%)	1 (2%)	3 (2%)
Diarrhoea	44 (9%)	18 (9%)	2 (4%)	13 (10%)
Stomatitis	14 (3%)	5 (3%)	1 (2%)	3 (2%)
Other gastrointestinal	16 (3%)	1 (1%)	0	1 (1%)
Skin	228 (48%)	60 (30%)	18 (40%)	32 (25%)
Pain	122 (26%)	28 (14%)	6 (13%)	16 (13%)
Cardiac	7 (1%)	3 (1%)	0	3 (2%)
Other non-haematological	34 (7%)	8 (4%)	2 (4%)	6 (5%)
Haematological§	124 (26%)	31 (16%)	6 (13%)	21 (18%)
Neutrophils	112 (24%)	25 (13%)	5 (11%)	15 (13%)
Platelets	21 (4%)	13 (7%)	3 (7%)	9 (8%)
Haemoglobin	2 (<1%)	2 (1%)	0	2 (2%)
Febrile Neutropenia	15 (3%)	2 (1%)	0	1 (1%)
Any toxic effect§	334 (71%)	104 (52%)	25 (54%)	62 (48%)

* Only the highest grade is counted and patients with more than one toxic effect of a particular grade were counted only once.
† Patients in the mitomycin/5-fluorouracil arm only were used for toxicity comparison.
‡ Numbers and percentages based on patients with submitted toxicity.
§ Patients with more than one toxic effect counted only once.

Table 1.
UK National Audit of anal cancer radiotherapy 2015 [18]. Reproduced with permission of Elsevier.

emission tomography (^{18}F FDG-PET), much improvement is made over the years in visualizing the primary tumor and involved regional lymph nodes and, hence, delineating GTV. In contrast, the estimation of microscopic metastatic disease remains poor and is largely based upon a few studies with documented locoregional recurrence in relation to tumor size and irradiated volumes [20–22]. The CTV for SCCA is notoriously complex, given the potential involvement of inguinal, iliac, mesorectal and presacral lymph nodes. Consensus contouring guidelines have been developed to assist radiation oncologists in setting up a treatment plan [23, 24]. With respect to the radiation dose, a two or three dose level for microscopic and macroscopic disease has emerged form clinical trials. For instance, in the Radiation Therapy Oncology Group (RTOG) 87-11 trial, a radiation dose of 30.6 Gy was given to the common iliac lymph nodes whereas a dose of 45 Gy was delivered to the lower iliac lymph nodes and 50.4 Gy to the primary tumor [25]. In contrast, in the United Kingdom Coordinating Committee on Cancer Research Anal Cancer Trial (UKCCCR-ACT) I and the European Organization For Research and Treatment of Cancer Radiotherapy (EORTC) 22861 trial the common iliac lymph nodes were not included in the elective radiation field, whereas a dose of 45 Gy was given to the lower iliac and inguinal lymph nodes with a boost to 60–65 Gy to the primary tumor [9, 10]. In the subsequent UKCCCR-ACT II the dose to the iliac and inguinal lymph nodes was limited to 30.6 Gy and the boost to the primary tumor to 50.4 Gy [26]. Despite these differences in radiation dose and volume, no striking difference in LRC was observed between these trials [9, 10, 25]. A number of retrospective studies have reported a better LRC with a higher radiation dose, at least in the locally advanced tumors [27–30]. This was confirmed in a systematic literature review [31] and a recent retrospective study from a large Scandinavian database [32]. However, in the French prospective randomized ACCORD-03 trial, which included only locally advanced cases, a marginal, non-significant increase in colostomy-free survival (CFS), a surrogate endpoint for LRC, was observed after 70 Gy, as compared with 60 Gy [33]. Consequently, in the absence of definitive evidence, current clinical guidelines do not advocate a higher radiation dose for larger tumors [34, 35].

2.1.2 The treatment gap

In the initial trials, a treatment gap of 6 weeks was included at an intermediate radiation dose [9, 10, 25]. This was done to allow for recovery from acute radiation toxicity, but also to give the tumor time to regress and to assess whether a radiation boost should be given with external beam irradiation or with brachytherapy. As results matured and further insight in tumor radiobiology was gained, this long treatment gap was considered to be potentially hazardous, due to the likelihood of tumor repopulation during the treatment gap. In the subsequent studies, the treatment gap was shortened to 2 weeks, which not only seemed to be feasible, but also resulted in better LRC in some studies [36–40] but not in others [41, 42]. With the advent of IMRT and VMAT, the entire radiation course could be administered without a treatment break. Today, most modern radiotherapy centers have implemented IMRT or VMAT for SSCA.

2.2 Chemotherapy and radiation for SCCA

2.2.1 Landmark studies

In June 1973, Dr. Nigro presented 3 cases with SCCA at a meeting of the American Proctologic Society in Detroit, that were treated with radiation therapy (RT) and concurrent Mitomycin C (MMC) and 5-fluorouracyl (5-FU) in a preoperative setting [7]. The rationale for this approach was to improve the LRC and overall survival (OS) of SSCA, since the results with radical surgery alone were modest, at best. Dr. Nigro realized that, in contrast with rectal cancer, SCCA originates from an organ which has an abundant lymphatic vessel supply, that allows rapid lymphatic tumor spread. In addition, there is limited space in the lower pelvis for radical surgery. The radiation dose was 30 Gy in 3–5 weeks via two large anterior-posterior opposed fields, and 30 mg of MMC was given on day 1 in a single bolus infusion and 1500 mg per day of 5-FU on days 2–6 in a continuous infusion. Six to 8 weeks later, two of them underwent an abdominoperineal resection, as planned. No tumor was found on microscopic examination of the operation specimen in these two cases. The third patient refused surgery and remained free of disease 1 year later [7]. This treatment regimen was expanded in a larger series, which confirmed the excellent results [43]. This pioneering work formed the basis for definitive CRT with higher, therapeutic radiation doses.

The superiority of this regimen compared with RT alone was established in two randomized phase III trials, the UKCCCR-ACT I and the EORTC 22861 [9, 10]. These trials were executed almost parallel in time and their design was strikingly similar, except for the eligibility criteria: in the EORTC trial only locally advanced patients were eligible, whereas in the ACT I all stages were accepted for inclusion. Despite this imbalance in patient selection, no major difference in the treatment outcome was observed between these two trials. Both studies showed a significant improvement in LRC control with CRT as compared with RT alone [9, 10]. In the ACT I, 3-year LRC increased from 47% after RT alone to 70% after CRT with concurrent 5-FU and MMC [9]. The corresponding figures in the EORTC 22861 trial were 55 and 68%, respectively [10]. The difference in LRC and progression-free survival (PFS) in the ACT I remained up to 12 years after treatment [44]. However, no difference in OS was found in either of these trials [10, 44].

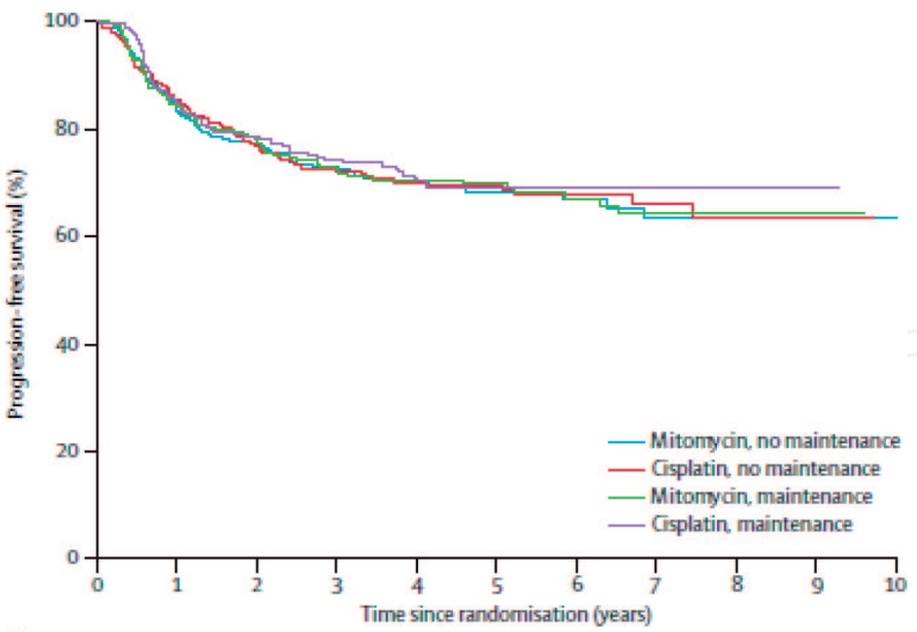
The value of MMC, in addition to 5-FU, was established in the phase III RTOG 87-04 study [25]. In this trial, however, MMC was given twice in the first and fifth week of the radiation treatment, as opposed to only once in the ACT I and EORTC 22861 trial. It resulted in considerably more grade 4-5 hematological toxicity than was seen in the European trials.

2.2.2 Subsequent pivotal studies

In the subsequent phase III RTOG 98-11 trial, the role of neo-adjuvant and concurrent cisplatin and 5-FU was addressed by comparing it with concurrent MMC and 5-FU [45]. While the combination of cisplatin and 5-FU was less toxic than MMC and 5-FU, the disease-free survival (DFS) and OS was significantly worse with the new regimen [46]. In the UKCCCR-ACT II, concurrent cisplatin, 5-FU and RT was compared with concurrent MMC, 5-FU and RT, with or without adjuvant cisplatin and 5-FU, in a 2 × 2 factorial design [26]. In this trial, which is the largest phase III trial carried out to date for anal cancer, no difference in PFS (**Figure 1**) and toxicity was observed between the four treatment arms [26]. The French phase III ACCORD 03 trial investigated the value of neo-adjuvant and concurrent cisplatin, 5-FU and RT, and radiation dose intensification, also in a 2 × 2 factorial design [33]. Whereas a marginal, non-significant increase in CFS was observed in the group that received the higher radiation dose, no difference in CFS was found between the patients with and without neo-adjuvant chemotherapy. Acute and late toxicity were similar between the four groups [33]. The EORTC 22011-40014 randomized phase II trial compared concurrent MMC, cisplatin and RT with MMC, 5-FU and RT [47]. The new combination proved to be highly effective, but more toxic, with a compliance of only 49% as opposed to 79% for the standard arm [47].

2.2.3 Variant schedules

In the UKCCCR-ACT I, EORTC 22861 and RTOG 87-04 trials, MMC was given once on day 1 [9, 10] or twice on day 1 and 29 of the radiation treatment [25],



Number at risk						
Mitomycin, no maintenance	223	266	117	48	15	0
Cisplatin, no maintenance	223	268	114	58	18	0
Mitomycin, maintenance	226	273	119	59	20	0
Cisplatin, maintenance	222	170	114	64	16	1

Figure 1.
MMC or cisplatin+5FU and radiation + or—adjuvant cisplatin/5-FU for SSCAC [26]. Reproduced with permission of Elsevier.

whereas 5-FU was administered in a continuous infusion day 1–4 or 5 and day 29–32 or 33. Variants of this treatment schedule have been explored with 5-FU given continuously in lower daily doses over the entire split-course radiation treatment [37], or by replacing 5-FU with capecitabine, an oral prodrug of 5-FU, given twice daily during the radiation treatment [48–51]. These schedules seemed feasible and equally effective as the standard schedule. In addition, capecitabine has the advantage of being able to be given on an outpatient basis.

Taken together, the original regimen of MMC and 5-FU remains the standard of care in CRT for SCCA, 45 years after its inception. There is a trend of using capecitabine instead of 5-FU because it is more patient friendly and equally effective. Arguably, MMC is more toxic than cisplatin in combination with 5-FU or capecitabine and RT [37], but this is dose dependent and seems to be equally effective in a single bolus of 10 mg/m^2 as 12 or 15 mg/m^2 or twice 10 mg/m^2 [9, 10, 25]. Furthermore, the combination of cisplatin and 5-FU is not more effective than MMC and 5-FU, but requires hospitalization for hydration procedures to prevent renal toxicity [26].

3. Prognostic factors in anal carcinoma

Well-known clinical prognostic factors in SCCA are age (>55 years better than ≤55 years), sex (female better than male), tobacco smoking (worse), primary tumor size and site (anal margin better than anal canal), T- and N-stage, tumor ulceration (worse if present) and histological differentiation grade [32, 52, 53]. Other prognostic factors include HPV-HR and certain genetic alterations.

3.1 Human papillomavirus

HPV-HR is causally related with the onset and progression of SCCA [5]. Once integrated into the host DNA, the main viral oncoproteins E6 and E7 interact with the tumor suppressor proteins p53 and retinoblastoma protein (pRb), respectively. P53 has a key role in maintaining DNA integrity, whereas pRb is a negative regulator of the cyclin-dependent kinase inhibitor p16. Upon persistent HPV-HR infection, p53 becomes permanently inactivated, disrupting DNA repair processes, and pRb inactivation induces upregulation of p16. As such, p16 is sometimes used as a surrogate marker of HPV-HR infection. These and other oncogenic processes lead to genomic instability, carcinogenesis and tumor progression. As a result, HPV-HR+ SCCA have a number of unique features, some of which have a prognostic or even a predictive value (**Figure 2**) [5].

Patients with HPV-HR+ SCCA have a significantly better outcome after CRT than HPV-HR-tumors [54–56]. Absolute difference in LRC/PFS varies from 32 to 67%, whereas the difference in OS varies from 22 to 52%. Interestingly, within the HPV-HR+ tumors, LRC and OS after CRT are significantly better in patients with tumors carrying a high HPV-HR DNA load than in those with a low HPV-HR DNA load [57]. Intratumoral p16 expression is also correlated with LRC and PFS after CRT for SCCA [58]. An even stronger discriminating effect on LRC and PFS is observed by combining p16 expression and HPV DNA tumor load [57].

P53 and p16 expression/HPV-HR+ are inversely correlated in SCCA [56, 58]. In addition, p53 expression and disruptive *TP53* mutations are associated with a significantly worse outcome after CRT [56, 58].

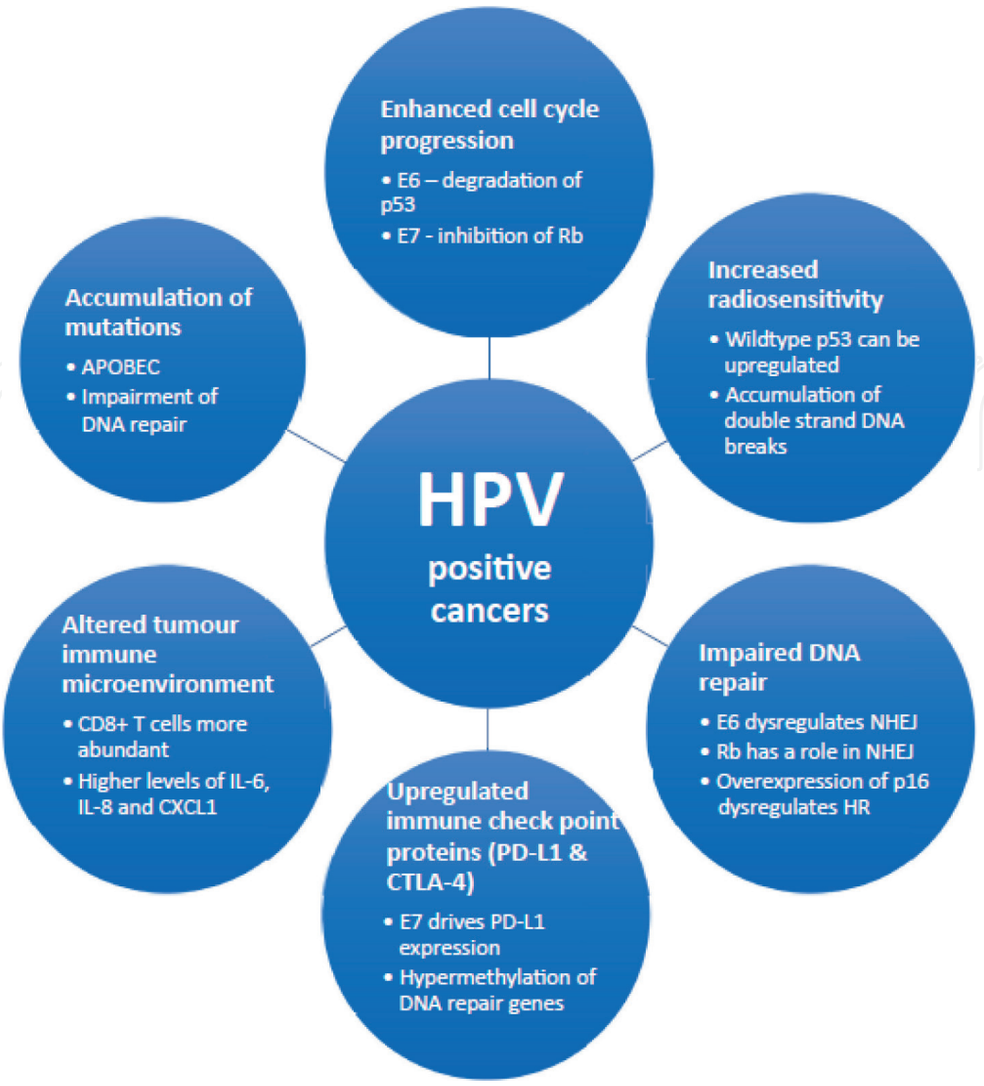


Figure 2.
Molecular features in HPV positive tumors [5]. Reproduced with permission of Elsevier.

3.2 Epidermal growth factor receptor

The epidermal growth factor receptor (EGFR) is frequently overexpressed in SCCA and this may confer a growth and survival advantage. In a subgroup analysis of the RTOG 98-11 trial, overexpression of EGFR and a downstream proliferation marker Ki67 was associated with a significantly worse DFS and OS [59]. In a recent small series of recurrent SCCA, high levels of alterations in the phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR) pathway, which is a growth and survival promoting pathway downstream of EGFR, were associated with poor OS [60].

3.3 Immune response

Persistent intratumoral HPV-HR infection can elicit a host immune response, which is mediated by immune checkpoint proteins such as cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and programmed cell death 1 (PD-1), expressed on activated T-cells and programmed cell death ligand 1 (PD-L1), expressed on tumors and various host cells [5, 61]. This can attract CD8+ T-lymphocytes into the tumor,

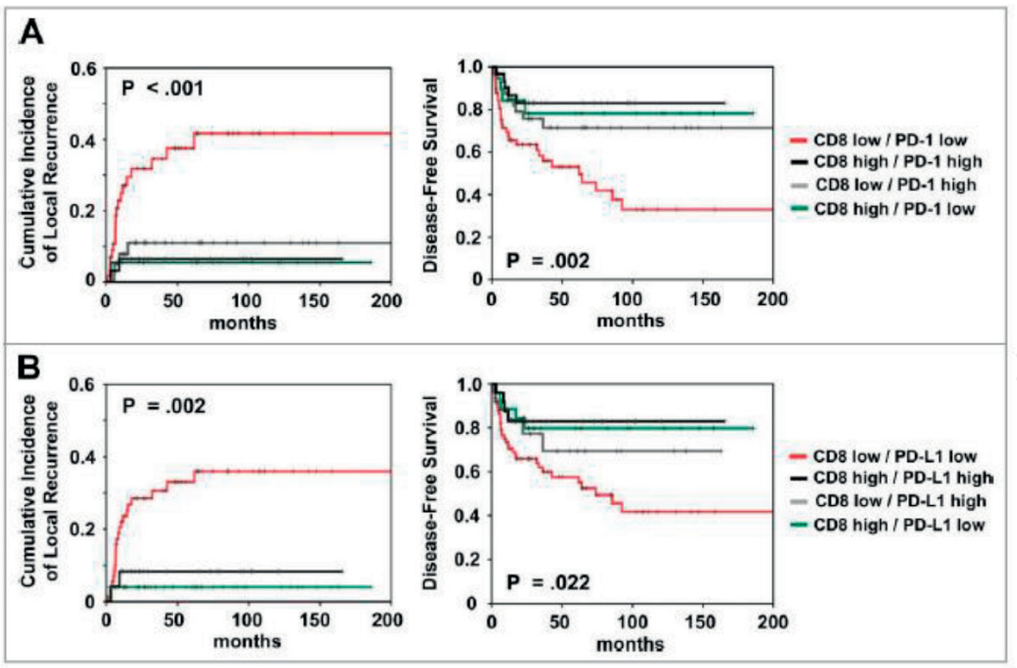


Figure 3.
Prognostic impact of CD8+/PD1 and DC8+/PD-L1 expression on LRC and DFS after CRT in SSCAC [62]. Reproduced with permission of Taylor and Francis.

so-called tumor-infiltrating lymphocytes (TILs). HPV-mediated intratumoral immune response has a significant influence on LRC and DFS, as illustrated by the amount of CD8+ TILs and PD-1 and PD-L1 expression levels after CRT in SCCA (**Figure 3**) [62].

4. Biological agents

Although the standard regimen of CRT with MMC and 5-FU is effective in SCCA, there is still room for improvement, in particular in the locally advanced cases and tumors that carry poor prognostic factors. Attempts have been made to investigate newer, promising agents. Here we focus on two avenues that have been explored.

Cetuximab is a chimeric IgG1 monoclonal antibody with a high affinity for EGFR. It has been tested in a few phase II trials in combination with concurrent CRT in SSCA, and turned out to be very toxic and probably also less effective than the standard regimen [63–67].

Two phase II trials have been published on the use of anti-PD-1 monoclonal antibodies in recurrent and/or metastatic SCCA, that is nivolumab [68] and pembrolizumab [69]. Objective responses were observed in 24 and 17%, respectively, and stable disease in 42% of the latter [68, 69]. Adverse events were acceptable.

5. The role of surgery in anal carcinoma

5.1 Salvage abdominoperineal resection

Radical surgery for SCCA is restricted to locoregional recurrent, non-metastasized and resectable tumors after CRT. The standard operation procedure is an abdominoperineal resection (APR), sometimes extended with resection of parts of the vagina or prostate, if involved, in order to obtain clear surgical margins [6]. This leaves a large pelvic floor defect, which preferably should be closed with a vertical

rectus abdominis myocutaneous flap (VRAM). Patients are left with a permanent colostomy. After APR, 5-year OS varies between 30 and 75%, depending upon whether or not clear resection margins have been obtained [6, 70]. Morbidity can be substantial, such as wound infections and poor healing of previously heavily irradiated organs and tissues. Wide resections into non-irradiated tissues and reconstructions with plastic flap techniques reduce these serious complications [6].

5.2 Curative local excision

A particular role for curative surgery in first line treatment of SCCA is reserved for small, T1N0 tumors of the anal margin, suitable for local excision (LE). This is not a trivial decision to make and these patients deserve to be seen by an experienced multidisciplinary team. Based on a recent pattern of care study in Australia, there is a wide variety in management of these small T1 tumors, depending upon the findings after a (non)excisional biopsy (**Figure 4**) [71]. In accordance with the guidelines and expert opinion, it is safe to say that T1N0 tumors < 1 cm, located in the anal margin, are good candidates for LE [34, 35]. This will probably account for only 4% of all anal cancers [72]. If pathological examination of the surgical specimen reveals that the resection is not radical, some form of additional treatment is warranted and should be discussed in a multidisciplinary team. If located in the anal canal, LE carries a risk of sphincter damage and is therefore relatively contraindicated. Nevertheless, a recent retrospective cohort study of

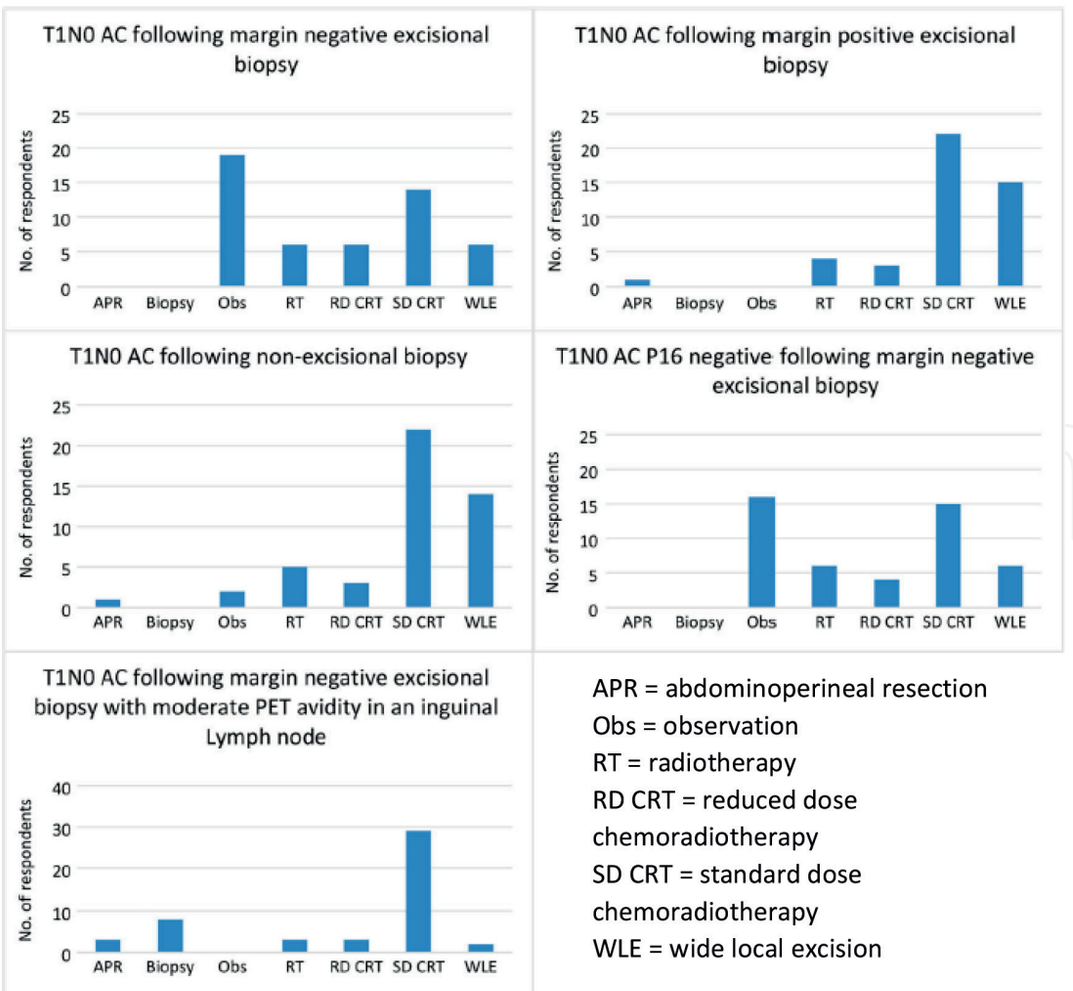


Figure 4.
Reported management of T1No anal cancer [71]. Reproduced with permission of Springer.

the US National Cancer Database on 2243 cases with T1 N0 SCCA has shown that over the period 2004–2012 LE was increasingly used in the more recent years, also for tumors of the anal canal [73]. Although criticized for its lack of information on the exact tumor location, LRC and DFS [74, 75], this study and the Australian survey [71] illustrate that clinicians are reluctant to treat these small tumors with standard CRT.

6. Treatment strategy

Today's clinical research on SCCA is focused on individualizing treatment as a function of estimated prognosis. A good example, for instance, is the UK trial "PersonaLising rAdioTherapy dOse in anal cancer" (PLATO), which offers a platform of 3 trials, ACT3, 4 and 5, for 3 different risk groups of SCCA [76].

ACT3 is a non-randomized trial for patients with low-risk T1N0 tumors of the anal margin, that undergo LE, followed by active surveillance if the resection margin is >1 mm. If the margin is ≤1 mm, postoperative reduced dose CRT is given locally (41.4 Gy in 23 fractions). In the Netherlands Cancer Institute, we use a somewhat different treatment policy for these tumors, taking a relatively new entity for SCCA into account, known as superficially invasive squamous cell carcinoma (SISCCA). SISCCA is defined as an invasive squamous cell carcinoma with an invasive depth of ≤3 mm and a horizontal spread of ≤7 mm that has been completely excised [77]. In the cervix, SISCCA is known to bear a minimal risk of microscopic lymph node metastasis and it is assumed to be similar for SISCCA of the anus, although the data supporting this are scarce [77]. We therefore have adopted a close surveillance policy for SISCCA of the anal margin. If the resection margin is too close or involved, a wider excision is performed, if possible. If not, postoperative reduced dose RT alone is given to the anus (45 Gy in 25 fractions). For T1N0 tumors that are microscopically >3 mm in invasive depth or >7 mm in horizontal spread, we also irradiate the inguinal lymph nodes to 45 Gy in 25 fractions. We do not advocate CRT in these cases, because the results with RT alone are excellent [35, 78, 79]. Furthermore, CRT is associated with an absolute increase of 9% of non-cancer related deaths compared with RT alone, mainly from cardiovascular cause and secondary tumors [44].

ACT4 is a randomized phase II trial for intermediate-risk tumors, T1–2 (≤4 cm) N0 or Nx, comparing LRC at 3 year after standard-dose CRT (50.4 Gy in 28 fractions) *versus* a reduced-dose CRT (41.4 Gy in 23 fractions). In the French guidelines, the advice for T1 and small T2 tumors is to treat them with RT alone [35]. In the Netherlands Cancer Institute, we follow the Dutch National guidelines, which advocate RT alone for T1N0 tumors and CRT for all other stages [80].

ACT5 is a randomized seamless pilot/phase II/phase III trial for high-risk SCCA, T1-2N1-3 or T3-4Nany, comparing 3-years' LRC after standard-dose CRT (53.2 Gy in 28 fractions) with that after 2 higher dose levels (58.8 and 61.2 Gy in 28 fractions) [76]. In the Netherlands Cancer Institute, we use CRT for these tumors with a relatively high radiation dose of 59.4 Gy in 30 fractions. We do not consider a lower radiation dose, because with VMAT the toxicity profile is acceptable [79].

7. Conclusions and future prospects

The treatment of SCCA has evolved over the last 5 decades from a mutilating radical surgical treatment with a modest survival probability to an individualized

radiation treatment with or without concurrent chemotherapy with good survival outcome and acceptable morbidity. Important improvements in radiation treatment techniques have been made, modern guidelines have been implemented and quality assurance is provided. However, there is still room for improvement. Quality of life analyses have infrequently been performed and are rarely taken into account in treatment decision making (e.g. [11, 81–83]). A good step forward in this respect is the development of a core outcome set of data, which should be the minimal information required in future clinical trials for anal cancer [84]. Radiation dose de-escalation and omitting concurrent chemotherapy for early tumors with good prognosis are important avenues to explore. On the other hand, new treatment modalities are needed for poor prognostic cases, such as HPV-HR negative SCCA. Immunotherapy seems to be a promising modality, either alone [68, 69] or in combination with chemotherapy [85]. Exploring the molecular profile of SCCA may reveal new potentially therapeutic targets and prognostic and predictive markers [60, 86, 87]. Circulating tumor DNA at baseline and in follow-up may become an important tool in treatment decision making [88]. These new insights and therapeutic avenues may eventually change the landscape of anal cancer treatment in the near future.

Conflict of interest

The authors have declared no potential conflict of interest.

Nomenclature

clinical target volume (CTV)	the microscopic tumor volume, based upon the estimated microscopic lymphatic tumor spread
CTLA-4	a member of the immunoglobulin superfamily, expressed on the cell surface of activated T-cells. It binds to B7-1 and B7-2 molecules of antigen presenting cells, which down-regulates the immune response, a process frequently occurring in cancer
3D-conformal radiotherapy (3D-CRT)	a 3-dimensional radiation treatment technique, which allows to shape the radiation dose distribution "conformal" to the shape of the planning target volume
epidermal growth factor receptor (EGFR)	a transmembrane protein, which is frequently overexpressed in a number of cancers. When activated, either by ligand binding (normal) or mutations (abnormal), it stimulates downstream signaling pathways, which promote DNA synthesis, cell growth and cell migration
gross tumor volume (GTV)	the macroscopic tumor volume as visualized with CT, MRI and/or PET
intensity-modulated radiotherapy (IMRT)	a refined version of 3D-conformal radiotherapy, where various segments within a radiation field allow to modulate the radiation fluency, in order to obtain conformity to irregularly shaped volumes

P16	a tumor suppressor protein, which slows down the cell cycle by inhibiting cyclin-dependent kinases
P53	a tumor suppressor protein, that plays an essential role in maintaining DNA integrity by various mechanisms. It can activate DNA repair proteins and induce cell cycle arrest to allow DNA repair, or, alternatively, initiate programmed cell death if DNA damage appears to be irreparable
PD-1	a member of the immunoglobulin superfamily, expressed on T-cells and pro-B-cells. It binds to PD-L1 on macro phages and dendritic cells, which down-regulates the immune system and promotes self-tolerance, a protective mechanism against auto-immune disease. PD-L1 is frequently overexpressed in many tumors, which promotes tumor tolerance
PI3K/AKT/mTOR pathway	an intracellular signaling pathway involved in cell cycle regulation. It is frequently overactive in many cancers, eliciting a growth and survival advantage
planning target volume (PTV)	the extension of CTV needed to account for systematic and random set up variation of the patient positioning
retinoblastoma protein (pRb)	a tumor suppressor protein, which prevents excessive cell growth by inhibiting DNA synthesis
volumetric modulated arc therapy (VMAT)	a refined version of IMRT, in which the radiation dose is delivered by rotating the gantry around the patient. The collimator head also rotates and contains moving leaves. The dose rate is also variable

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