Treatment outcomes and prognostic factors after chemoradiotherapy for anal cancer

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Abstract

Background: Squamous cell carcinoma of the anus (SCCA) is a rare malignancy with rising incidence, associated with human papilloma virus (HPV). Chemoradiotherapy (CRT) is the preferred treatment. The purpose was to investigate treatment failure, survival and prognostic factors after CRT.

Material and methods: In this prospective observational study from a large regional centre, 141 patients were included from 2013 to 2017, and 132 were eligible for analysis. The main inclusion criteria were SCCA, planned radiotherapy, and performance status (ECOG) ≤2. Patient characteristics, disease stage, treatment, and treatment response were prospectively registered. Disease-free survival (DFS), overall survival (OS), and locoregional treatment failure after CRT were analysed. Hazard ratios (HRs) were estimated with Cox`s proportional hazards model.

Results: Median follow-up was 54 (range 6–71) months. Eighteen patients (14%) had treatment failures after CRT; of these 10 (8%) had residual tumour, and 8 (6%) relapse as first failure. The first treatment failure was locoregional (11 patients), distant (5 patients), and both (2 patients). Salvage abdomino-perineal resection was performed in 10 patients, 2 had resections of metastases, and 3 both. DFS was 85% at 3 years and 78% at 5 years. OS was 93% at 3 years and 86% at 5 years. In analyses adjusted for age and gender, HPV negative tumours (HR 2.5, p=0.024), N3 disease (HR 2.6, p=0.024), and tumour size ≥4 cm (HR 2.4, p=0.038) were negative prognostic factors for DFS.

Conclusion: State-of-the-art chemoradiotherapy for SCCA resulted in excellent outcomes, and improved survival compared with previous national data, with <15% treatment failures and a 3-year DFS of >80%.

Background

Squamous cell carcinoma of the anus (SCCA) is a rare malignancy with increasing incidence in several countries, also in Norway [1, 2]. In 2018 ninety new cases in Norway [3] and 48541 worldwide [4] were registered. A paradigm shift in the treatment of anal cancer came in the early 1970s, when Nigro *et al* described the significant effect of chemoradiotherapy (CRT) [5]. Randomized studies conducted during the 1990s demonstrated that CRT with 5-fluorouracil (5-FU) and mitomycin C (MMC) was superior to radiotherapy (RT) alone or CRT with 5-FU [6-8]. Later randomized studies found that MMC and 5-FU were superior to cisplatin and 5-FU, and the latter gave no benefit as induction or maintenance treatment [9-11]. The Norwegian national guidelines recommend MMC combined with 5-FU or capecitabine to radiation doses up to 54–58 Gy [12]. The optimal radiation dose remains undefined; however for most SCCA it is likely to be greater than 50.4 Gy [13]. Studies have shown that intensity modulated radiation therapy (IMRT) with simultaneous integrated boost (SIB) is effective and results in less acute adverse effects compared to 3D conformal radiotherapy [14, 15]. Guidelines recommend adhering to a maximal treatment time, because prolonged treatment time is associated with detrimental outcome [16].

Even though outcomes are generally good, there is a risk of locoregional and/or distant treatment failure. In a previous large Nordic patient series including 1266 patients, there was a 17% locoregional failure rate (LFR), and 11% distant failure rate (DFR) [17]. Among Norwegian patients, 13% had residual tumour and after salvage surgery, primary treatment control was obtained in 93%. However, 24% developed relapse, where most were locoregional [18]. The recommended treatment for locoregional failure is salvage abdomino-perineal resection (APR) [13], with a 3-year overall survival (OS) of >50% [19, 20].

Several clinical prognostic factors for outcome after CRT have been described. Nodal involvement, skin ulceration, male gender, and tumour size >5 cm have been found to be associated with inferior outcome in randomized studies [21, 22]. In addition, HPV status is an important

prognostic factor [23, 24], and systematic reviews and meta-analyses have shown that HPV infection and/or p16 overexpression is associated with improved survival outcomes [25, 26]. In patients with p16 positive tumours, tumour-infiltrating lymphocytes were associated with better outcome [27]. Additional prognostic factors include pre-treatment positron emission tomography (FDG PET/CT) parameters [28], and leukocytosis and neutrophilia [29].

Treatment of SCCA is centralised in Norway, and all patients in our health region, comprising 3 million people, are treated at Oslo University Hospital (OUS). Studies on population-based, unselected large cohorts can demonstrate the efficacy of modern CRT in a real-world population. Establishing prognostic factors is important to identify high-risk patients who may need more intensive treatment regimens or closer surveillance. Although CRT in general has shown good effect, previous national data showed high treatment failure rates [18], and we sought to investigate the outcomes after modern CRT. The aim of this study was to investigate disease-free survival (DFS), OS, and LFR, in unselected patients treated at OUS with curative intent, and to investigate prognostic factors.

Material and methods

The present study is the "Anal Cancer Radiotherapy – Prospective study of treatment outcome, patient-reported outcomes, utility of imaging and biomarkers, and cancer survivorship (ANCARAD)", a prospective multi-disciplinary observational trial (NCT1937780). The main inclusion criteria were histologically proven SCCA, planned RT, and adequate performance status (ECOG ≤2). Eligible patients were approached between October 2013 and September 2017 at OUS, and a total of 141 patients were included. Patient characteristics and details of disease and treatment were registered, including disease stage, tumour size determined by clinical and radiological information, details of magnetic resonance imaging (MRI) and PET when available, radiotherapy and chemotherapy given, surgery, and treatment response and survival. Patients attended follow-up for

5 years after treatment. All initial biopsies were re-examined by an expert pathologist for classification of morphology, differentiation grade, HPV status, T-cell infiltration and presence of pushing border. The ANCARAD study was approved by the Regional Ethical Committee South-East (2012/2274) and the OUS Institutional Study Board. All patients signed informed consent.

Patients had pelvic MRI, CT thorax/abdomen/pelvis, and as advised in the study protocol also FDG PET/CT before treatment for staging according to tumour (T), nodes (N), metastasis (M) using TNM classification 7th Edition [30]. All patients were discussed in a multidisciplinary team (MDT) meeting, and treated according to current national guidelines at the time.

The contouring for radiotherapy was mostly based on the Australasian guidelines [31]. The gross tumour volume (GTV) included the primary anal tumour and lymph node metastases. The clinical target volume (CTV) encompassed GTV with a 1–2 cm margin, and elective lymph nodes, typically perirectal, internal iliac, in some cases external iliac and inguinal lymph node regions [32] . The cranial border of the CTV was at the bifurcation of the common iliac artery, however for patients with T1–T2N0 tumours localized in the anal canal/margin the cranial border was at the lower end of the sacroiliac joint. An additional 1 cm in all directions was added to the CTV to obtain the planning target volume. At the time of study start, the standard radiotherapy technique was 3D conformal radiotherapy, but by the end of the study IMRT or volumetric modulated arc therapy (VMAT) was the standard technique, with either sequential boost or SIB. The recommended GTV dose was 54 Gy for T1–2N0 tumour (low-risk group) and 58 Gy for T3–T4 or N1–3 tumour (high-risk group). Lymph node metastases received 54 Gy if <2 cm and 58 Gy if >2 cm, and the elective CTV dose was 46 Gy. Radiotherapy was delivered in 2 Gy fractions, either sequentially with 1.8–2.0 Gy fractions, or with SIB. Radiation was given 5 days per week, and measures were taken to deliver treatment within recommended maximal treatment time (41/44 days).

Concomitant chemotherapy was delivered with MMC 10 mg/m^2 (max 20 mg) IV day 1 and 5-FU 1000 mg/m^2/day as a continuous infusion on days 1–4. 5-FU could be replaced by capecitabine 825 mg/m^2 twice daily on all days of radiotherapy. MMC was replaced with cisplatin in a few cases, in

particular for patients with oligometastatic disease. Patients with T1–2N0 cancer received one cycle of chemotherapy during radiotherapy week 1, and patients with T3–4 or N1–3 cancer received an additional cycle week 5.

The patients underwent a clinical examination 6 weeks after completed CRT to evaluate tumour response and side effects of treatment. At 3 months after CRT patients underwent a clinical examination including anoscopy/proctoscopy, MRI of the pelvis, and either FDG PET/CT or CT thorax/abdomen/pelvis. Those with partial response were followed further until 6 months after treatment. Patients who did not obtain complete response, had progressive disease, or later developed recurrence, were considered for salvage surgery. Patients with complete response entered a 5-year follow-up program including clinical examination and CT/MRI imaging.

HPV status was detected with *in situ* hybridization on tumour tissue sections for HPV 16, 18, 31, 33, 35, 45, 52, 56, 58 and 66 using INFORM HPV III Family 16 Probe. T-cell infiltration and pushing border were assessed visually by microscope, however most tissue samples were biopsies and not surgically removed lesions, therefore this evaluation was not always assessable and the results should be interpreted with caution. Tumour size was set as the largest tumour diameter. Surgically removed lesions with malignancy were registered with a lesion diameter as specified in the pathology description, however one patient had a lesion only described as small which was registered as 5 mm.

The primary endpoint of the study was 3-year DFS. Secondary endpoints included complete tumour regression, local recurrence, 5-year DFS, OS, and the association between tumour stage, histopathological features, and recurrence/survival. The endpoints were coherent with a later consensus of a core outcome set for clinical trials of CRT for anal cancer [33].

Statistics

The first day of RT was the starting point for calculating time to event for survival analyses and follow-up. Time to treatment failure for patients who did not obtain complete response was set to 3 months from first day of radiotherapy. Locoregional and/or distant relapses were registered.

Salvage treatment included surgery, chemotherapy and radiotherapy. N-status (N0–2 vs N3) and T-status (T1–2 vs T3 and T4) were pooled when analysing prognostic factors due to small patient numbers. Tumour localization was divided into two groups, with anal margin only as one group. Due to large number of groups in the classification of morphology we divided the groups in two; basaloid versus other. Second cancer diagnosis includes previous and simultaneous cancer. Survival patterns were analysed by the Kaplan-Meier method (censoring date March 31, 2020). Prognostic factors were analysed by Cox' proportional hazard model. The assumption of proportionality was checked by visual inspection of log-log plots. Unadjusted analyses and analyses adjusted for age and gender were performed. Unadjusted analyses were done for gender, age, smoking, HPV, T-cell infiltration, histology, pre-treatment stoma, differentiation grade, TNM, second cancer diagnosis, tumour diameter, tumour localization and pushing border. Adjusted analyses were performed for factors statistically significant at the 5% level in unadjusted analyses. Hazard ratio (HR) and Confidence intervals (CI) are given. Analyses were performed using SPSS version 25.0.

Results

Of 141 patients included in the study, 9 patients were excluded due to metastatic disease (6), surgery instead of CRT (1), not verified cancer (1), or withdrawn consent (1), leaving 132 patients who received CRT and were eligible for final analysis. Median age was 63 years (range 39–90), 73% were women, 19% had a previous or simultaneous other cancer, 42% had T3–T4 tumours, 45% had N1–3 disease, and 80% had HPV positive tumour (Table 1). Two patients had M1 disease with lymph nodes that could be included in the radiation field (one para-aortic and one by the common iliac artery) and received curatively intended CRT. Median follow-up was 54 (range 6–71) months.

CRT was delivered with conventional radiotherapy in 33%, and IMRT or VMAT in 67% of the patients (Table 2). The median dose to tumour was 54 Gy for low-risk cancers (T1–T2N0) and 58 Gy for high-risk cancers (T3–4N0/T1–4N+). Treatment was given within the recommended time period

for 80% of all patients (90% of low-risk and 73% of high-risk patients), and 10% discontinued treatment. The main reasons for treatment break/discontinuation were toxicity, infection (i.e abscess, pneumonia) or stoma placement.

Chemotherapy was delivered to 122 patients, while 10 (8%) patients were not considered eligible for chemotherapy due to age or comorbidity. All patients completed the first cycle. The main reasons for primary dose reduction were infection, high age, or large intestinal volumes in the radiation field. For patients with chemotherapy discontinuation, the main reasons were infection, bone marrow depression, stomatitis, mucositis, dermatitis and enteritis (Table 2).

In all, 121 (92%) patients had complete response after CRT, one (0.8%) died before evaluation, while 10 patients (8%) had residual tumour and/or metastases. Eight patients (6%) later developed locoregional or distant recurrence; hence 18 (14%) patients had treatment failures (Table 2). The first treatment failure was locoregional in 11 patients, distant in five and both locoregional and distant in two patients. Thus, the LFR was 10%. Among patients with treatment failures, seven (39%) had HPV negative tumour, 16 (89%) initially had high-risk disease, and six (33%) did not complete RT within the recommended time period. For the 8 patients who initially had complete response and later relapse, median time to recurrence was 22 months (range 16–47). Salvage APR was performed in 13 patients a median 11 months (range 7–48) after start of CRT, whereof 3 patients also had resection of metastases, and an additional two patients had metastasectomy. A wide resection was necessary to obtain free resection margins and 10 patients had a vertical rectus abdominis muscle flap and one a gracilis flap. R0 resection was obtained in 9 patients and 4 patients had R1 resection. Following APR, three patients later developed distant metastasis. Chemotherapy and/or RT were delivered as treatment to six patients, and some patients received several types of treatment modalities (Table 2). Eleven of the 18 patients with treatment failure were alive with no evidence of disease at last follow-up, with a median follow-up time of 45 months after their last surgery.

DFS at 3 and 5 years was 85% and 78%, respectively (Figure 1A). OS was 93% at 3 years and 86% at 5 years (Figure 1B). Three-year DFS was 79% in patients with high-risk disease and 94% in patients with low-risk disease (Figure 1C), and 3-year OS was not significantly different between the two patient groups (Figure 1D). Patients with male gender, age ≥70 years, T3 or T4 tumour, N3 disease, low differentiation grade, and HPV negative tumours seemed to have worse DFS, however only statistically significant for HPV negative tumours (Figure 2A-F). No significant survival differences were observed between patients treated with conventional RT versus IMRT/VMAT (data not shown). There was a non-significant trend of an association between stage and DFS, as shown in Supplementary Figure 1.

In unadjusted analysis HPV negative tumour, N3 disease and tumour size ≥4 cm were statistically significant negative prognostic factors for DFS (Table 3). These factors remained significant after adjusting for age and gender; HPV negative tumours (HR 2.5, CI 1.1–5.6, p=0.024), N3 disease (HR 2.6, CI 1.1–6.0, p=0.024) and tumour size ≥4 cm (HR 2.4, CI 1.1–5.5, p=0.038) (Table 3). For OS, HPV negative tumour was the only statistically significant negative prognostic factor in unadjusted analysis (HR 2.8, CI 1.0–7.8, p=0.044), after adjusting for age and gender the association was reduced (HR 2.4, CI 0.9–6.8, p=0.095) (Table 3). We investigated if the effect of HPV was different in men and women, however no significant interaction was seen for DFS (p=0.50) or OS (p=0.92). Sensitivity analyses performed by excluding the two patients with oligometastatic disease showed the same significant prognostic factors with similar hazard ratios.

When only investigating high-risk patients, we found HPV, differentiation grade and second cancer diagnosis to be prognostic factors for DFS. These factors remained statistically significant in analyses adjusted for age and gender with HPV negativity (HR 3.4, CI 1.4–8.7, p=0.009), moderate differentiation grade (HR 0.3, CI 0.1–0.8, p=0.018) and second cancer diagnosis (HR 3.3, CI 1.4–8.0, p=0.008). For OS, second cancer diagnosis (HR 3.7, CI 1.1–11.9, p=0.030) was the only statistically significant prognostic factor in analysis adjusted for age and gender.

Discussion

After modern CRT in our institution including dedicated follow-up and advanced salvage surgery for residual tumour or recurrence, there was a low rate of treatment failures (14%) and high survival rates with 3-year DFS of 85% and OS 93% for all patients. Three-year DFS was 79% for patients with high-risk disease and 94% for patients with low-risk disease. Even among patients with treatment failure, salvage surgery resulted in good long-term outcomes, advocating a radical treatment approach. These outcomes compare favourably with those reported in a review of randomized and retrospective studies where 3-year DFS rates exceeded 80% in T1–2N0 patients and was 60% or lower in patients with locally advanced disease [34]. The survival rates are also better than those previously reported in Norway [18]. This is in line with the results from Sekhar *et al* demonstrating considerable decline in loco-regional treatment failure to 16% and improved 5-year OS to 76% in a large UK institute over the last 25 years [35]. Thus, contemporary treatment outcomes have improved compared to those in historic trials.

The chemotherapy regimen is roughly the same as in previous studies with MMC/5-FU delivered concomitantly with RT. However, radiation doses vary considerably between studies. Some studies used a lower dose, and others delivered a boost several weeks after completed CRT [8, 11]. Treatment gaps in RT have today been largely abandoned [16, 36], and the importance of overall treatment time has been demonstrated. Our patients received a relatively high radiation dose to tumour (median 54 Gy for low-risk and 58 Gy for high-risk disease). Tumour control probability modelling studies suggest that a higher radiation dose is associated with better local tumour control in high-risk patients. Muirhead *et al* suggested a 2-year improvement of local control rate from 50 to 80% by escalating dose from 50 to 55 Gy in late stage tumours [37]. Johnsson *et al* showed that higher RT dose was associated with higher local tumour control probability [38]. In that study, it was suggested that addition of chemotherapy may correspond to a dose of 5–10 Gy, and that men may

need higher dose than women for tumour control. These studies, in addition to our results, suggest that radiation doses are important for local tumour control, in particular for locally advanced tumours. The challenge remains to determine the optimal RT dose for different tumour stages, and to identify patients in need of improved CRT efficacy. The ongoing PLATO study investigates the role of de-escalating RT doses in early stage from 50.4 Gy in 28 fractions to 41.4 Gy in 23 fractions, and escalating RT doses in locally advanced anal cancers from 53.2 Gy in 28 fractions to 58.8 Gy or 61.6 Gy in 28 fractions [39]. Pre-treatment FDG PET/CT uptake volumes have been suggested as a prognostic factor [28] and may also identify potential areas for further dose escalation in clinical trials [40].

Increased biological understanding is necessary for a personalised approach to anal cancer, for stratification in clinical trials, and to identify potential biomarkers [41] . Checkpoint inhibition has shown promising efficacy in metastatic anal cancer [42]. There is a biologic rationale to complement CRT with immunotherapy in anal cancer, in particular in HPV positive patients with high levels of tumour-infiltrating lymphocytes [43]. The potential role of immunotherapy combined with CRT is currently explored in clinical trials.

Although intensified treatment likely improves oncological outcomes, this must be balanced against long-term side effects of CRT. In previous publications from Norway, detrimental side effects on anorectal function and health-related quality of life (HRQOL) were observed [44, 45]. A recent systematic review identified HRQOL concerns, in particular reduced bowel function and sexual problems [46]. Several aspects of toxicity and of life impact, including HRQOL, are identified as core outcome measures after CRT for anal cancer [33]. IMRT may be beneficial for reducing long-term toxicity, as suggested by a recent UK national cohort showing moderate patient-reported symptoms 1 year after IMRT [47]. Prospective clinical trials should aim at assessing long-term toxicity and HRQOL after modern CRT.

The observed LFR of 10% was lower in the present study compared with previous Norwegian population-based results, and the improvement is probably not related to radiation dose, since this

was more or less similar in both series [18]. However, in the present study modern contouring guidelines were used, imaging with MRI and PET was improved, and most patients were treated with VMAT/IMRT. Delineation quality has proven to be a prognostic factor in anal cancer [48]. Also, the previous chemotherapy regimen for locally advanced tumours in Norway was neoadjuvant and concomitant cisplatin/5-FU, while patients in the present study received concomitant MMC and 5-FU which has shown to be superior [10, 11]. Staging has also improved, as MRI has been implemented as standard for all patients, and in the present study most patients also had a FDG PET/CT. Better staging modalities are believed to cause nodal stage migration, but may also result in over-staging [49]. In the previous Norwegian study, treatment was given at 5 different hospitals [18], while in this study all patients were treated at a single institution; however national guidelines should ensure similar treatment.

N3 stage, HPV negative tumours, and tumour size ≥4 cm were significant negative prognostic factors, as has been described in earlier studies [17, 21-26, 28]. The prognostic effect of N3 stage and tumour size and the worse DFS and OS for locally advanced tumours suggest treatment stratification [50]. Also, some centres and the ongoing PLATO trial use tumour size as a criterion for treatment stratification and treat patients with tumour ≥4 cm with the same CRT regimens as T3 tumours [39]. Worse overall survival according to stage was also found by Dahl *et al* [51]. Although survival was not significantly worse in men in the present study, previous studies have shown that men have worse prognosis [3, 18, 21, 22]. In addition to these features pre-treatment FDG PET/CT uptake intensity and volumes are predictive for locoregional failure [28]. In this study, all diagnostic biopsies were classified by one pathologist; however details such as pushing border or morphology did not convey further prognostic information. Tumour infiltrating leukocytes (TIL) have been reported to have prognostic impact in HPV positive tumours [27], however this was not demonstrated in the present study, likely due to small sample size.

We found that among patients with locally advanced tumours, a previous or synchronous second cancer diagnosis was a significant prognostic predictor for DFS and OS. These patients are often excluded from clinical trials. The high rate of previous cancer is in line with results in a previous Nordic publication where 15.5% had a history of previous malignancy, and significantly more patients had cancer in the cervix uteri, vulva, and lung than expected [17]. This is likely related to common etiological factors such as HPV infection and smoking.

Although this is a relatively large patient series for a rare cancer, the number of patients in subgroups is low leading to wide confidence intervals and challenges regarding inclusion of covariates in the survival models. A limitation is the non-randomized study design with no new intervention. The major strength of this study is the prospective data with comprehensive detailed registration of patient, tumour, histopathology, treatment, and outcomes. Future trials should focus on improved treatment stratification, and elucidating the possible role of checkpoint inhibition.

Conclusion

We present here improved population-representative outcome data after modern chemoradiotherapy for anal cancer. Patients had excellent outcomes in terms of high DFS and low locoregional treatment failure rates. HPV negativity, large tumour size, and extensive nodal involvement were negative prognostic factors.

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Disclosure of interest

The authors report no conflicts of interest.

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Figure legends

Figure 1. Survival after chemoradiotherapy for anal cancer, A) Disease-free survival all patients, B) Overall survival all patients, C) Disease-free survival high-risk versus low-risk patients, D) Overall survival high-risk versus low-risk patients.

Figure 2. Disease-free survival (DFS) after chemoradiotherapy for anal cancer. A) DFS female versus male patients, B) DFS by age groups, C) DFS by T stage, D) DFS by N stage, E) DFS by pathological differentiation grade, F) DFS by HPV-status.

Table 1. Patient and tumour characteristics

Patient characteristics	N (%)		
	All patients (n=132)	**Low-risk (n=52) T1–2N0**	**High-risk (n=80) T(any)N+ or T3–4N0**
Age (years)			
Median (range) years	63 (39–90)	62 (39–81)	63.5 (40–90)
Gender			
Female	96 (72.7%)	37 (71.2%)	59 (73.8%)
Male	36 (27.3%)	15 (28.8%)	21 (26.3%)
Current smoking			
Yes	41 (31.1%)	18 (34.6%)	23 (28.7%)
No	86 (65.2%)	32 (61.5%)	54 (67.5%)
Unknown	5 (3.8%)	2 (3.8%)	3 (3.8%)
Known HIV-status			
Positive	3 (2.3%)	2 (3.8%)	1 (1.3%)
Previous or simultaneous cancer*			
All	25 (18.9%)	8 (15.4%)	17 (21.3%)
Gynaecological cancer	7 (28%)	2 (25%)	5 (29.4%)
Breast cancer	6 (24%)	2 (25%)	4 (23.5%)
Lung cancer	4 (16%)	2 (25%)	2 (11.8%)
Skin cancer	4 (16%)	1 (12.5%)	3 (17.6%)
Gastrointestinal cancer	2 (8%)	1 (12.5%)	1 (5.9%)
Prostate cancer	2 (8%)	-	2 (11.7%)
Kidney cancer	1 (4%)	-	1 (5.9%)
Lymphoma	1 (4%)	-	1 (5.9%)
HPV high risk status**			
Positive	106 (80.3%)	42 (80.8%)	64 (80.0%)
Negative	25 (18.9%)	9 (17.3%)	16 (20.0%)
Unknown	1 (0.8%)	1 (1.9%)	0 (0%)
Organ transplant			
Yes	1 (0.8%)	0 (0%)	1 (1.3%)
Pre-treatment stoma			
Yes	13 (9.8%)	2 (3.8%)	11 (13.8%)
No	117 (88.6%)	50 (96.2%)	67 (83.8%)
Stoma placement during treatment	2 (1.5%)	0 (0%)	2 (2.5%)
Tumor localization			
Anal margin only	11 (8.3%)	9 (17.3%)	2 (2.5%)
Anal canal and/or anal margin and/or rectum	121 (91.7%)	43 (82.7%)	78 (97.5%)
T stage			
T1	15 (11.4%)	12 (23.1%)	3 (3.8%)
T2	61 (46.2%)	40 (76.9%)	21 (26.3%)
T3	25 (18.9%)	–	25 (31.3%)
T4	31 (23.5%)	–	31 (38.8%)
Largest tumor diameter			
Median (range) mm	39 (5–110)	30 (5–49)	52.5 (11–110)
N stage			
N0	73 (55.3%)	52 (100%)	21 (26.3%)

	N1	14 (10.6%)	–	14 (17.5%)
	N2	23 (17.4%)	–	23 (28.7%)
	N3	22 (16.7%)	–	22 (27.5%)
M stage				
	M0	130 (98.5%)	52 (100%)	78 (97.5%)
	M1***	2 (1.5%)	0 (0%)	2 (2.5%)
Morphology				
	Large-cell keratinizing	56 (42.4%)	19 (36.5%)	37 (46.3%)
	Large-cell non-keratinizing	26 (19.7%)	8 (15.4%)	18 (22.5%)
	Basaloid	20 (15.2%)	9 (17.3%)	11 (13.8%)
	Mixed type	23 (17.4%)	9 (17.3%)	14 (17.5%)
	Verrucous	6 (4.5%)	6 (11.5%)	0 (0%)
	Unclassified	1 (0.8%)	1 (1.9%)	0 (0%)
Differentation grade				
	Well	13 (9.8%)	9 (17.3%)	4 (5.0%)
	Moderate	94 (71.2%)	32 (61.5%)	62 (77.5%)
	Poor	18 (13.6%)	8 (15.4%)	10 (12.5%)
	Unknown	7 (5.3%)	3 (5.8%)	4 (5.0%)
T-cell infiltration				
	Yes	30 (22.7%)	15 (28.8%)	15 (18.8%)
	No	81 (61.4%)	29 (55.8%)	52 (65.0%)
	Unknown/not assessable	21 (15.9%)	8 (15.4%)	13 (16.3%)
Pushing border				
	Yes	13 (9.8%)	6 (11.5%)	7 (8.8%)
	No	89 (67.4%)	36 (69.2%)	53 (66.3%)
	Unkown/Not assessable	30 (22.7%)	10 (19.2%)	20 (25.0%)

*Simultaneous (n=6), simultaneous and previous (n=1), or previous cancer (n=18). Two patients had two additional cancer diagnoses.

** HPV 16, 18, 31, 33, 35, 45, 52, 56, 58 and 66.

*** Lymph node metastases (paraaortic, common iliac) included in radiation field.

Table 2. Details of radiotherapy and chemotherapy, treatment response after CRT, and treatment failures.

n = 132			**n (%)**
Radiotherapy			
Technique			
	Conventional radiotherapy		43 (32.6%)
	Intensity modulated radiation therapy (IMRT)		12 (9.1%)
	Volumetric modulated arc therapy (VMAT)		77 (58.3%)
Boost			
	Sequential boost		118 (89.4%)
	Simultaneous integrated boost		14 (10.6%)
Mean dose to tumour			56.1 Gy
Radiotherapy treatment time, mean days (range)			38 (16-55)
Treatment completion			
	Within recommended time		105 (79.6%)
	Prolonged time		14 (10.6%)
	Discontinued		13 (9.8%)
Pause in radiotherapy but completed within recommended time			15 (11.4%)
Chemotherapy			
Concomitant chemotherapy*			
	Mitomycin (MMC) + 5-fluorouracil (5-FU) one cycle		58 (43.9%)
	MMC + 5-FU two cycles		59 (44.7%)
	Other		2 (1.5%)
	Neoadjuvant and concomitant cisplatin + 5-FU and/or MMC + 5-FU		3 (2.3%)
	No chemotherapy		10 (7.6%)
Chemotherapy treatment discontinued**			16 (12.1%)
Chemotherapy dose reduction			15 (11.4%)
Treatment response and treatment failure			
Complete response and no subsequent treatment failure			114 (86.4%)
First treatment failure			18 (13.6%)
	Residual tumour/ metastases at response evaluation		10 (7.6%)
		Locoregional	6 (4.5%)
		Locoregional and distant	2 (1.5%)
		Distant metastases	2 (1.5%)
	Recurrence		8 (6.1%)
		Locoregional recurrence	5 (3.8%)
		Distant metastases	3 (2.3%)
Second treatment failure			3 (2.3%)
	Locoregional		1 (0.8%)
	Distant		2 (1.5%)
Treatment of failure***			
	Surgery		15 (11.4%)
		Abdomino-perineal resection	13 (9.8%)
		Resection of metastasis	5 (3.8%)
	Chemotherapy		6 (4.5%)
	Radiotherapy		2 (1.5%)
	None		2 (1.5%)

*MMC + capecitabine (n=1). MMC omitted in second cycle (n=5).
Other: 5-FU monotherapy (n=1), 2 cycles of cisplatin + 5-FU (n=1).

**Patients planned for 2 cycles, but second cycle was interrupted/discontinued. Four patients also had dose reduction.

***Some patients received several treatment modalities.

Table 3. Unadjusted analyses and analyses adjusted for age and gender for disease-free survival and overall survival.

			Disease-free survival						Overall survival					
			Unadjusted analyses			Analyses adjusted for age and gender			Unadjusted analyses			Analyses adjusted for age and gender		
Variabel		**n**	**HR**	**95% CI**	**P-value**	**HR**	**95% CI**	**P-value**	**HR**	**95% CI**	**P-value**	**HR**	**95% CI**	**P-value**
Gender	Female (ref)	96	1.0						1.0					
	Male	36	2.2	0.9-4.8	0.05				2.1	0.7-5.8	0.170			
Age	< 70 years (ref)	102	1.0						1.0					
	≥ 70 years	30	1.6	0.7-3.5	0.273				2.4	0.9-6.5	0.075			
Current smoking	No (ref)	86	1.0						1.0					
	Yes	41	1.2	0.5-2.6	0.736				1.1	0.4-3.3	0.875			
HPV high-risk	Positive (ref)	106	1.0						1.0					
	Negative	25	2.9	1.2-6.3	0.008	2.5	1.1-5.6	0.024	2.8	1.0-7.8	0.044	2.4	0.9-6.8	0.095
T-cell infiltration	No (ref)	81	1.0						1.0					
	Yes	30	1.2	0.5-2.9	0.714				1.1	0.4-3.7	0.834			
Morphology	Basaloid type (ref)	20	1.0						1.0					
	Other types	111	0.9	0.2-2.4	0.848				1.0	0.3-3.6	0.980			
Pre-treatment stoma	No (ref)	117	1.0						1.0					
	Yes	13	0.9	0.2-3.0	0.801				1.5	0.4-5.8	0.528			
Differentiation grade	Poor (ref)	18	1.0						1.0					
	Moderate	94	0.5	0.2-1.4	0.212				0.9	0.2-4.3	0.936			
	Well	13	0.5	0.1-2.3	0.342				1.3	0.2-9.1	0.805			
T-Stage	T1/T2 (ref)	76	1.0						1.0					
	T3	25	2.1	0.9-5.2	0.105				1.1	0.3-4.0	0.940			
	T4	31	1.5	0.6-3.8	0.388				1.6	0.5-5.0	0.395			
N-stage	N0-N2 (ref)	110	1.0						1.0					
	N3	22	2.6	1.1-5.9	0.026	2.6	1.1-6.0	0.024	2.4	0.8-6.8	0.113			
M-stage	M0 (ref)	130	1.0						1.0					
	M1	2	3.7	0.5-27.6	0.205				0.1	NA*	0.689			
Second cancer	No (ref)	107	1.0						1.0					

diagnosis	Yes	25	2.1	0.95-4.8	0.066				2.2	0.8-6.1	0.129			
Largest tumour diameter	< 4 cm (ref)	66	1.0			1.0			1.0					
	≥ 4 cm	66	2.5	1.1-5.7	0.032	2.4	1.1-5.5	0.038	1.6	0.6-4.4	0.374			
Tumour localization	Anal margin only (ref)	11	1.0						1.0					
	Anal canal and/or anal margin/rectum	121	2.8	0.4-20.4	0.320				23.2	NA*	0.421			
Pushing border	No (ref)	89	1.0						1.0					
	Yes	13	0.5	0.1-2.3	0.390				1.0	0.2-4.4	0.955			

*NA due to small number of patients.

Figure 1

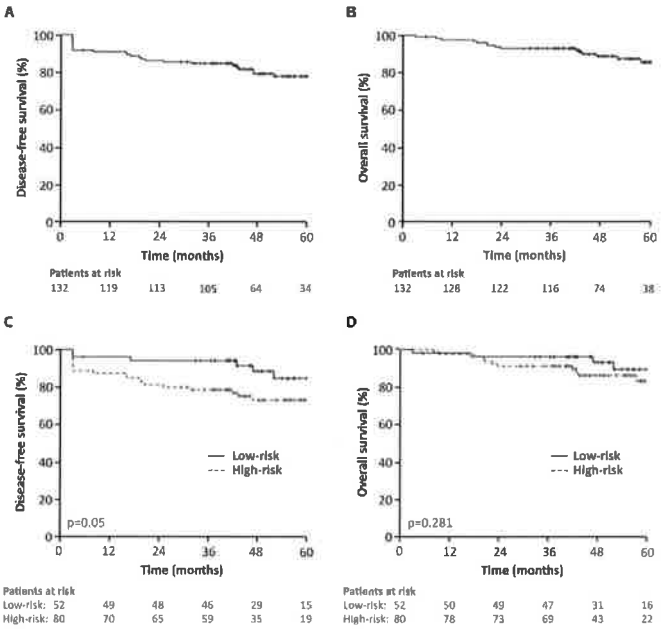

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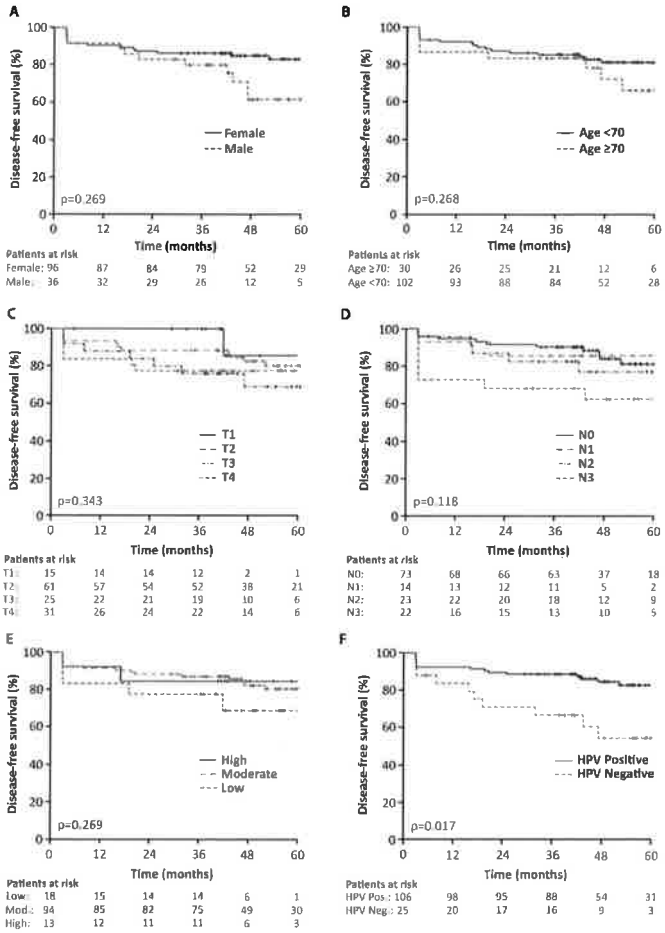
Figure 2



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