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Chemoradiotherapy with capecitabine for locally advanced anal carcinoma: an alternative treatment option

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Background: Capecitabine is an established treatment alternative to intravenous 5-fluorouracil (5-FU) for patients with rectal cancer receiving chemoradiotherapy. Its place in the treatment of locally advanced anal carcinoma (AC), however, remains undetermined. We investigated whether capecitabine is as effective as 5-FU in the treatment of patients with locally advanced AC.

Methods: One hundred and five patients with squamous cell AC stage T2-4 (T2 > 4 cm), N0-1, M0 or T1-4, N2-3, M0, were included in this retrospective study. Forty-seven patients were treated with continuous 5-FU (750 mg m^{-2}) on days 1–5 and 29–33, mitomycin C (MMC, 10 mg m^{-2}) on day 1, and radiotherapy; 58 patients were treated with capecitabine (825 mg m^{-2} b.i.d. on weekdays), MMC (10 mg m^{-2}) on day 1, and radiotherapy. The primary end points of the study were: clinical complete response rate, locoregional control (LRC) and overall survival (OS). Secondary end points were: colostomy-free survival (CFS), toxicity and associations of genetic polymorphisms (*GSTT1*, *GSTM1*, *GSTP1* and *TYMS*) with outcome and toxicity.

Results: Clinical complete response was achieved in 41/46 patients (89.1%) with 5-FU and in 52/58 patients (89.7%) with capecitabine. Three-year LRC was 76% and 79% ($P = 0.690$, log-rank test), 3-year OS was 78% and 86% ($P = 0.364$, log-rank test) and CFS was 65% and 79% ($P = 0.115$, log-rank test) for 5-FU and capecitabine, respectively. *GSTT1* and *TYMS* genotypes were associated with severe (grade 3–4) toxicity.

Conclusions: Capecitabine combined with MMC and radiotherapy was equally effective as 5-FU-based chemoradiotherapy. This study shows that capecitabine can be used as an acceptable alternative to 5-FU for the treatment of AC.

Anal carcinoma (AC) is a relatively rare malignancy with an annual incidence of ~1 in 100 000 in European countries (Netherlands Cancer Registry). Treatment of locally advanced disease evolved from abdominoperineal resection to sphincter-preserving radiotherapy by the late 1970s. It was subsequently shown by Nigro *et al* in 1983, and later confirmed in two pivotal randomised controlled trials (RCTs), that radiotherapy with concomitant 5-fluorouracil (5-FU) and mitomycin C (MMC) resulted in superior disease control compared with radiotherapy alone (Nigro *et al*, 1983; UKCCCR Anal Cancer Trial Working Party, 1996; Bartelink *et al*, 1997). Nowadays, the standard of care is full-dose radiation therapy combined with 5-FU, administered as a continuous infusion for 4 or 5 days in week 1 and 5 of radiotherapy, and MMC as a bolus on day 1 (James *et al*, 2013).

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Radiotherapy is usually applied using a two- or three-field technique to a total dose of 45–50.4 Gy in 4–5 weeks, sometimes followed by a boost up to 59.4 Gy (UKCCCR Anal Cancer Trial Working Party, 1996; Bartelink *et al*, 1997; Gunderson *et al*, 2012) or, more recently, as intensity-modulated radiation therapy (IMRT) (Pepek *et al*, 2010; Bazan *et al*, 2011).

Capecitabine is an oral 5-FU pre-prodrug, which offers an alternative to 5-FU that does not require inpatient hospital care, is more convenient for patients and reduces the costs of treatment. In addition, by administering capecitabine on all radiation days, a longer duration of exposure to 5-FU and its cytotoxic metabolites during irradiation can be achieved, thereby potentially increasing the radiosensitising effect.

In a recent non-inferiority study, the efficacy of capecitabine in the neoadjuvant treatment with chemoradiation of locally advanced rectal cancer has been demonstrated (Hofheinz *et al*, 2012). In AC, however, RCTs are difficult to perform because of its low incidence and a relatively low failure rate. Indeed, capecitabine-based chemoradiation in AC has been investigated in only a few small studies (Glynne-Jones *et al*, 2008; Deenen *et al*, 2013).

To investigate the effectiveness of capecitabine, we performed a retrospective study to determine clinical complete response (cCR) rate, locoregional control (LRC) and overall survival (OS) in consecutive patients treated with capecitabine, MMC and IMRT, and compared outcomes with patients treated with 5-FU-based chemoradiotherapy.

MATERIALS AND METHODS

Patient characteristics. All consecutive patients, $\geqslant 18$ years of age, with histologically confirmed locally advanced squamous cell AC, classified as T2-4 (with T > 4 cm), N0-1 and M0, or T1-4 with N2-3 and M0) treated at our institute between August 2003 and August 2011 with concurrent chemoradiotherapy were included. Patients with a history of other malignancies (except resectable basal cell or squamous cell carcinoma of the skin), patients with recurrent disease at presentation and patients receiving chemotherapy other than a fluoropyrimidine + MMC were excluded. Disease staging was performed according to the American Joint Committee on Cancer staging manual (6th edition) and the International Union Against Cancer system. The study was approved by the institutional ethics committee.

Treatments

Radiotherapy. Patients treated before March 2006 received three-dimensional conformal radiotherapy (CF-RT), whereas subsequent patients received simultaneous integrated boost IMRT. With CF-RT, the primary tumour and elective pelvic and inguinal lymph nodes (LNs) were irradiated to a dose of 45 Gy (25 fractions of 1.8 Gy). After a planned rest period of 3 weeks, a boost of 8–11 fractions of 1.8 Gy was delivered to the primary tumour and macroscopically involved LNs. The number of fractions of the boost (8 in case of cCR, 11 in case of partial response) was determined by digital rectal examination (DRE), and MRI of the pelvis, if required, in week 5. Patients treated with IMRT received a total dose of 59.4 Gy (33×1.8 Gy), delivered on weekdays in 6.5 consecutive weeks, without planned treatment break. Pelvic and inguinal LNs were electively irradiated to a total dose of 49.5 Gy (33×1.5 Gy). Here also, the decision to give an additional boost of 3×1.8 Gy was based on DRE and pelvic imaging, if required, in week 5.

Chemotherapy. Between August 2003 and January 2008, chemotherapy consisted of 5-FU in all patients, given as a continuous infusion of 750 mg m^{-2} on days 1–5 (in week 1) and days 29–33 (in week 5) of radiation treatment. In February 2008, a phase I dose-escalation study was initiated using capecitabine instead of 5-FU (Deenen *et al*, 2013). From February 2008 onward, all patients received capecitabine, except six patients who were not included in the study and received 5-FU (this included three HIV-positive patients and one patient considered unable to comply with instructions for taking oral medication). Patients received 825 mg m^{-2} capecitabine b.i.d. on radiation days, except during the three boost fractions. Patients with a body surface area (BSA) > 2.0 m^{-2} were dosed according to BSA 2.0 m^{-2}. All patients received 10 mg m^{-2} MMC as an intravenous bolus injection on day 1, with a maximum of 15 mg. The durations of unscheduled treatment interruptions for radiotherapy and chemotherapy were recorded, as were the reasons for deviating from the treatment protocol.

Toxicity evaluation. During chemoradiotherapy, acute toxicity was recorded and discussed weekly during the multidisciplinary treatment discussion. Acute toxicity was assessed retrospectively within four domains (dermatological, gastrointestinal, haematological and genitourinary) according to the NCI-CTCAE, v3.0. Toxicities were scored as worst grade occurring from start of treatment until 30 days after the last fraction of radiotherapy.

Response evaluation and follow-up. Tumour response was evaluated by DRE and palpation of inguinal nodes during treatment, at the end of treatment, and 4–6 weeks after completion of treatment. Clinical complete response was defined as complete resolution of palpable tumour by physical examination. Patients were included for evaluation of clinical response if there was at least 12 weeks of follow-up available. Follow-up evaluation at the outpatient clinic included physical examination and laboratory analysis, including squamous cell carcinoma antigen as a tumour marker, and was performed every 3 months during the first 2 years after treatment, every 6 months in the third year and once a year thereafter. In case of suspected recurrence, additional imaging and histological confirmation were performed.

Pharmacogenetics. Polymorphisms in the gene encoding thymidylate synthase (*TYMS*) and in genes encoding glutathione S-transferase enzymes have been associated with outcome and toxicity in patients treated with fluoropyrimidines and radiotherapy (Pullarkat *et al*, 2001; Ambrosone *et al*, 2006; Mahimkar *et al*, 2011). We analysed associations with response and toxicity for the following polymorphisms: *GSTT1* (deletion), *GSTM1* (deletion), *GSTP1* 313A > G, *TYMS* 3′UTR 6-bp ins/del and *TYMS* 5′UTR variable number of 28-bp tandem repeats (VNTR). With regard to the *TYMS* VNTR polymorphism, patients were categorised as having low expression (*2/*2, *2/*3C or *3C/*3C) or high expression genotypes (*2/*3G, *3C/*3G or *3G/*3G) based on the G > C SNP in the second repeat (Mandola *et al*, 2003). Polymorphisms in *GSTT1* and *GSTM1* were determined by polymerase chain reaction (PCR) and visualisation of PCR products on agarose gel, *GSTP1* 313A > G was determined using a commercial real time PCR assay and polymorphisms in *TYMS* were assessed by PCR and sequencing (primer sequences available on request).

End points and statistical considerations. The primary end points were cCR rate, LRC and OS. Colostomy-free survival (CFS) and acute toxicity were secondary end points. Baseline patient and disease characteristics were compared using Student's *t*-test, Mann–Whitney *U*-test, Fisher's exact test or χ^2 whenever appropriate. The Kaplan–Meier method was used to determine LRC, OS and CFS. Time to locoregional failure was defined as the interval between treatment day 1 and the day on which clinical signs of progression at the primary site or regional LNs (inguinal or pelvic) first occurred. Time to colostomy was defined as the interval between treatment day 1 and the day of surgery for colostomy. Pretreatment colostomies were considered tumour-related

colostomies at $t=0$. Pretreatment colostomies that were reversed during follow-up were ignored and not considered to be an event in the analysis. A colostomy was classified as treatment-related if it was performed either during chemoradiotherapy or after the completion of therapy, in absence of histologic evidence of disease. Overall survival was calculated from the first treatment day till the day of death. Patients that did not experience an event were censored at the day of last follow-up. Groups were compared using log-rank tests. Fisher's exact test was used to assess toxicity between groups as a dichotomised outcome (none or grade 1–2 toxicity *vs* grade $\geqslant 3$ toxicity). All statistical tests were two-sided with significance set at $P<0.05$. All analyses were performed using SPSS 16.0 (SPSS Inc, Chicago, IL, USA).

RESULTS

Patient and treatment characteristics. A total of 129 AC patients were identified, 63 were treated with 5-FU and 66 with capecitabine. Sixteen patients within the 5-FU group were excluded, for the following reasons: metastatic disease (7), concurrent other malignancy (3), recurrent disease at presentation (3), patient record not available (2) and treatment with cisplatin (1). Eight patients were excluded from the capecitabine group, for: metastatic disease (5), recurrent disease (2) and concurrent other malignancy (1). The first 18 patients within the capecitabine group were treated in a phase I study that was reported previously (Deenen *et al*, 2013). Seven of these patients, which were included in the analysis, received a dose lower than 825 mg m^{-2} bid (500–650 mg m^{-2} bid). There were no significant differences between groups in baseline patient and disease characteristics (Table 1). One patient in the capecitabine group was identified with a heterozygous *DPYD*2A* mutation and was treated with a 50% reduced dose of capecitabine.

Compliance with treatment plan. All patients completed radiotherapy. Radiotherapy was completed without interruptions in 43/47 (92%) of the patients in the 5-FU group and in 55/58 (95%) of the patients in the capecitabine group. Planned chemotherapy was completed without interruptions in 45/47 (96%) of the patients in the 5-FU group and in 50/58 (86%) of the patients in the capecitabine group. Delays lasted between 11–12 days with 5-FU (median: 11.5 days) and 1–14 days with capecitabine (median: 3 days). Of the planned cumulative dose, all patients in the 5-FU group received 100%; in the capecitabine group patients received on average 95% ($\pm$15%).

Toxicity evaluation. Acute toxicity tended to be more prevalent in the capecitabine group (Table 2), with grade $\geqslant 3$ radiation dermatitis occurring significantly more often with capecitabine than with 5-FU. In the 5-FU group, the incidence of grade $\geqslant 3$ radiation dermatitis was not affected by radiation technique (13% for both 5-FU/CF-RT and 5-FU/IMRT). Grade 4 toxicity occurred in 2/47 patients (4%) that were treated with 5-FU (both haematological toxicities), leading to delay of chemo- and radiotherapy in one case. Grade 4 toxicities occurred in 5/58 patients (9%) treated with capecitabine (two dermatological, two haematological, and one gastrointestinal toxicity), leading to the delay of chemo- and radiotherapy in two cases (the other toxicities occurred at the end of the treatment period or before a weekend break). No toxic deaths were observed.

Response evaluation. All patients except one were considered for response evaluation (one patient in the 5-FU group was lost to follow-up 11 weeks after end of treatment). In the 5-FU group 41/46 patients (89%) and in the capecitabine group 52/58 patients (90%) reached a cCR, at a median of 3 weeks (range: -2–22) and 3 weeks (range: -3–28) after the last treatment day, respectively. When calculated from the first day of treatment, patients in the capecitabine group reached cCR earlier than patients in the 5-FU group (69 days *vs* 93 days, $P=0.015$, Mann–Whitney *U*-test). Characteristics of the patients that did not reach cCR are summarised in Table 3. Rates of cCR did not differ significantly between 5-FU/CF-RT, 5-FU/IMRT, and capecitabine/IMRT subgroups (88%, 87%, 90%, respectively; $P=0.926$).

Survival parameters. With a median duration of follow-up of 49 months (range: 4–96) in the 5-FU group and 23 months (range: 13–54) in the capecitabine group, LRC did not differ between groups; 3-year LRC rates were 76% (95% CI: 60%–92%) and 79% (95% CI: 57%–101%) for 5-FU and capecitabine, respectively ($P=0.690$, Figure 1A). The 3-year CFS was 65% (95% CI: 44%–86%) and 79% (95% CI: 56%–102%) for 5-FU and capecitabine, respectively ($P=0.155$, Figure 1B). Four pretreatment colostomies in the 5-FU group and one in the capecitabine group were reversed during follow-up. Treatment-related colostomies occurred in the 5-FU group in four cases (9%) and in the capecitabine group in one case (2%). Overall surival was not significantly different between groups, 3-year OS was 78% (95% CI: 64%–92%) and 86% (95% CI: 68%–104%) for 5-FU and capecitabine, respectively ($P=0.364$, Figure 1C). There were no significant differences between the 5-FU/CF-RT, 5-FU/IMRT, and capecitabine/IMRT groups with regard to LRC and OS (Figure 2A and B). Pairwise comparisons showed that the 5-FU/IMRT and capecitabine/IMRT groups were not significantly different with regard to LRC and OS ($P=0.577$ and $P=0.809$, respectively). Comparisons with the 5-FU/CF-RT group also showed no significant differences (data not shown).

Pharmacogenetics. Table 4 shows the associations of polymorphisms with clinical response and toxicity. No associations with response were observed. However, the *TYMS* VNTR polymorphism was associated with severe toxicity; 40% of the patients with a low expression genotype experienced grade 3–4 toxicity *vs* 18% of the patients with a high expression genotype. When different types of toxicity were considered separately (Table 5), patients with the low expression genotype more often experienced severe dermatological, gastrointestinal, genitourinary toxicity, although the differences for the individual toxicities did not reach statistical significance. The *GSTT1* NULL genotype also tended to be associated with increased overall toxicity. There was a significant association between the *GSTT1* NULL genotype and dermatological toxicity; 43% of these patients experienced severe dermatological toxicity, compared with 19% of the patients without the NULL genotype ($P=0.040$, Fisher's exact test).

DISCUSSION

We show in a cohort of consecutively treated patients with locally advanced AC that, in combination with full-dose radiation therapy, comparable cCR rate, LRC, and OS can be achieved with capecitabine as with 5-FU. The cCR rate ($\sim$90%), 3-year LRC (75%–80%), and 3-year OS (80%–85%) compare favourably with other studies (UKCCCR Anal Cancer Trial Working Party, 1996; Bartelink *et al*, 1997; James *et al*, 2013). Capecitabine was given on all radiation days, thereby achieving a longer duration of interaction between radiosensitising chemotherapy and radiation. All patients completed radiotherapy, and on average 95% of the planned dose of capecitabine could be administered.

Although the incidence of severe toxicity was generally low, grade 3–4 dermatological toxicity was with 31% far more frequent in patients treated with capecitabine and radiotherapy than in patients treated with 5-FU and radiotherapy (13%). Most likely this is due to longer duration of combined exposure to chemotherapy and radiation with bi-daily capecitabine, and not due to differences

Table 1. Patient and treatment characteristics

	5-FU + MMC ($n = 47$)		Capecitabine + MMC ($n = 58$)		
Characteristic	**No.**	**%**	**No.**	**%**	***P*-value**
Age (years), median (range)	53.5 (36.8–83.8)		59.3 (41.3–86.4)		0.277
Gender					
Male	23	49	22	38	0.322
Female	24	51	36	62	
T-classification					
T1	1	2	0	0	0.837
T2	20	43	29	50	
T3	18	38	19	33	
T4	8	17	10	17	
N-classification					
N0	22	47	18	31	0.103
N1	13	28	19	33	
N2	9	19	9	16	
N3	3	6	10	17	
Nx	0	0	2	3	
UICC stage					
Stage 0	0	0	0	0	0.221
Stage I	0	0	0	0	
Stage II	17	36	14	24	
Stage III	30	64	42	72	
Stage IV	0	0	0	0	
Not known	0	0	2	3	
Primary tumour site					
Anal canal	40	85	50	86	0.957
Anal margin	5	11	5	9	
Both	2	4	3	5	
HIV status					
Negative	12	26	27	47	0.078
Positive	7	15	4	7	
Unknown	28	60	27	47	
SCC tumour marker					
Normal ($<2.0\,\mu g\,l^{-1}$)	24	51	36	62	0.223
Elevated ($\geqslant 2.0\,\mu g\,l^{-1}$)	22	47	19	33	
Unknown	1	2	3	5	
***DPYD**2A genotype**					
Wild type	7	15	56	97	1.000
Heterozygous	0	0	1	2	
Unknown	40	85	1	2	
Radiation technique					
CF-RT	24	51	0	0	—
IMRT	23	49	58	100	
Radiation dose					
Surdosage given?	5-FU + CF-RT	5-FU + IMRT	Capecitabine + IMRT		
Yes	24 (100%)	19 (83%)	32 (55%)		—
No	0 (0%)	4 (17%)	26 (45%)		
Total radiation dose to primary tumour, median (range)	64.8 (64.8–66.6)	64.8 (59.4–68.4)	64.8 (59.4–70.2)		—
Total radiation dose to LNs, median (range)	64.8 (45.0–66.6)	54.9 (49.5–58.5)	54.9 (49.5–60.3)		—

Abbreviations: 5-FU = 5-fluorouracil; CF-RT = three-dimensional conformal radiotherapy; HIV = Human immunodeficiency virus; IMRT = intensity-modulated radiation therapy; LNs = lymph nodes; MMC = mitomycin C; SCC = squamous cell carcinoma.

Table 2. Acute toxicity according to treatment group

	5-FU + MMC ($n = 47$)		Capecitabine + MMC ($n = 58$)		
Type of toxicity	**No.**	**%**	**No.**	**%**	***P*-value[a]**
Dermatological toxicity					
No toxicity	0	0	0	0	0.035
Grade 1–2	41	87	40	69	
Grade 3–4	6	13[b]	18	31	
Gastrointestinal toxicity					
No toxicity	17	36	4	7	1.000
Grade 1–2	29	62	52	90	
Grade 3–4	1	2	2	3	
Haematological toxicity					
No toxicity	7	15	7	12	1.000
Grade 1–2	37	79	47	83	
Grade 3–4	3	6	3	6	
Genitourinary toxicity					
No toxicity	34	72	28	48	0.586
Grade 1–2	11	24	29	50	
Grade 3–4	2	4	1	2	

Abbreviations: 5-FU = 5-fluorouracil; CF-RT = three-dimensional conformal radiotherapy; IMRT = intensity-modulated radiation therapy; MMC = mitomycin C.
[a]Fisher's exact test for no toxicity or grade 1–2 toxicity *vs* grade 3–4 toxicity.
[b]Grade 3–4 dermatological toxicity was equally frequent in 5-FU/CF-RT and 5-FU/IMRT subgroups, with a 13% incidence in both groups.

Table 3. Characteristics of patients without clinical complete response

	5-FU + MMC ($n = 5$)		Capecitabine + MMC ($n = 6$)		
Characteristic	**No.**	**%**	**No.**	**%**	***P*-value**
Age (years), median (range)	65.6 (36.8–73.5)		56.2 (41.3–65.1)		0.545
Gender					
Male	3	60	5	83	0.559
Female	2	40	1	17	
T-classification					
T1	0	0	0	0	0.177
T2	2	40	0	0	
T3	2	40	3	50	
T4	1	20	3	50	
N-classification					
N0	2	40	1	17	0.086
N1	1	20	0	0	
N2	2	40	2	33	
N3	0	0	3	50	
HIV status					
Negative	0	0	4	67	0.333
Positive	1	20	1	17	
Not known	4	80	1	17	
Primary tumor site					
Anal canal	6	100	6	100	1.000
Anal margin	0	0	0	0	
Both	0	0	0	0	

Abbreviations: 5-FU = 5-fluorouracil; LN = lymph node; MMC = mitomycin C.

in radiation technique/schedule, as in the 5-FU group there was no effect of radiation technique on the incidence of grade 3–4 dermatological toxicity. In another study, in which capecitabine was combined with conventional radiotherapy, and applied without a treatment gap as in this study, a comparable rate of grade $\geqslant 3$ dermatological toxicity (38%) was found (Glynne-Jones *et al*, 2008). Late toxicity was not taken into account in this study and deserves attention in future studies. An important question is how the biological effects of bi-daily capecitabine relate to those of a schedule in which 5-FU is given in week 1 and 5. To our knowledge, there are no studies comparing tissue levels of active metabolites of 5-FU (e.g. FdUMP) after continuous infusion of 5-FU compared with bi-daily capecitabine. For several reasons, however, it is likely that cumulative exposure of tumour to 5-FU's active metabolites is at least as high with bi-daily capecitabine at $825\,mg\,m^{-2}$ on weekdays as with continuously infused 5-FU at $750\,mg\,m^{-2}$ for 5 days in week 1 and 5. First, the dose of capecitabine that is used is at or close to the maximum tolerable dose (Glynne-Jones *et al*, 2006; Deenen *et al*, 2013). And, while after administration of 5-FU, the relative exposure of normal and tumour tissue to 5-FU is equal (Kovach and Beart, 1989), after administration of capecitabine exposure to 5-FU was found to be higher in tumour than in adjacent healthy tissue, in colorectal tumours (Schüller *et al*, 2000). In addition, there is preclinical evidence that radiation combined with capecitabine (and not with 5-FU) has synergistic antitumour activity due to upregulation of thymidine phosphorylase (which converts 5′-deoxy-5-fluorouridine into 5-FU) by irradiation, theoretically leading to higher concentrations of 5-FU in tumour tissue (Sawada *et al*, 1999). Importantly, the cumulative dose of capecitabine that is used, relative to 5-FU in the traditional schedule, is in the same range as the cumulative dose of capecitabine relative to 5-FU in the neoadjuvant treatment of rectal cancer (Hofheinz *et al*, 2012).

In an exploratory pharmacogenetic analysis, we showed that the low expression *TYMS* VNTR genotype was associated with higher rates of severe toxicity. A recently published large clinical study and meta-analysis confirms the validity of this association (Rosmarin *et al*, 2014). We also found that the *GSTT1* NULL genotype was associated with severe dermatological toxicity. This may be explained by the role of glutathione S-transferase enzymes in counteracting radiation-induced oxidative stress, and is in line with previous reports (Yoon *et al*, 2011). We did not confirm our previous observation of the *GSTP1* 313A > G polymorphism being associated with response (Deenen *et al*, 2013).

Several important limitations of this study should be mentioned. The sample size does not permit to statistically demonstrate non-inferiority of capecitabine to 5-FU. Owing to the low incidence of AC and a low failure rate after chemoradiotherapy, a non-inferiority study would be very difficult to undertake. For this reason, treatment decisions will need to be based on retrospective studies and institutional experiences such as presented here. Our patient groups were treated serially in time and we cannot rule out improvement of health care during this time period. However, the time frame in which patients were treated is relatively small, and all patients were treated by a multidisciplinary team that discusses the patients weekly. We therefore assume that the quality of medical care did not change to the extent that it would confound the results of the study. Although we considered all consecutively treated patients, some selection bias might have been introduced by

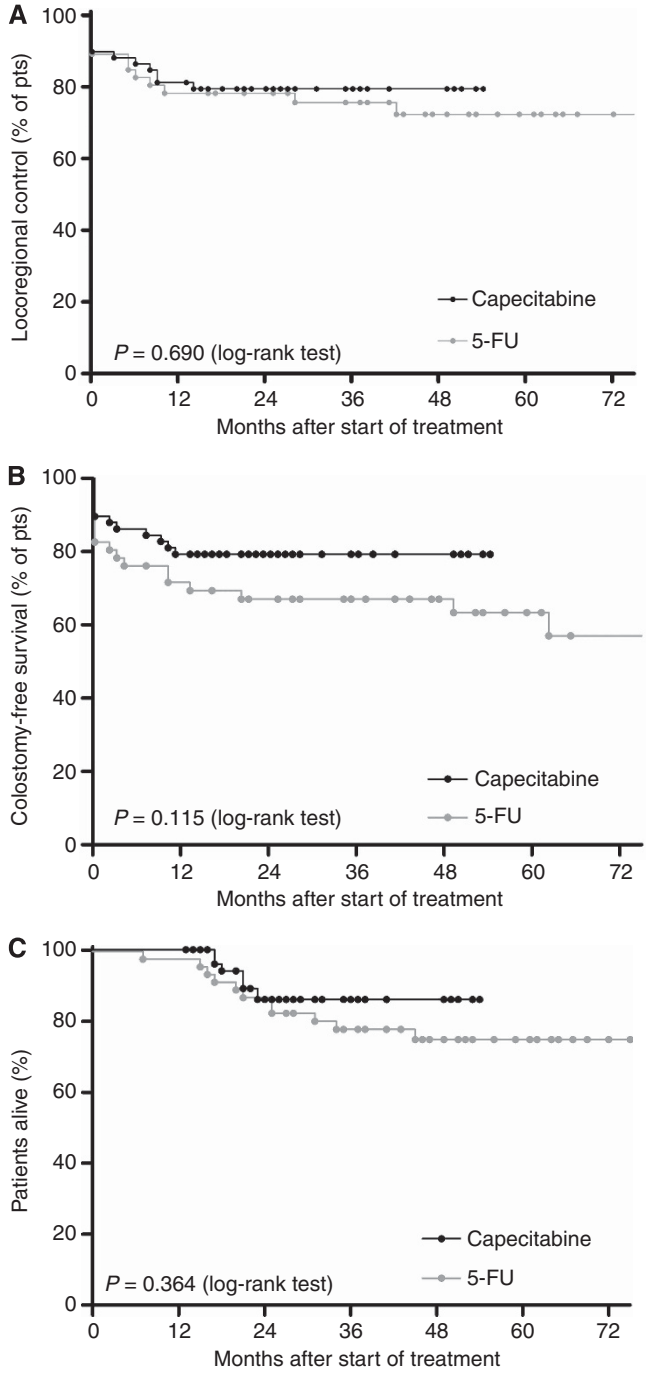

Figure 1. Outcome of patients treated with either 5-FU or capecitabine-based chemoradiotherapy. The figures show the locoregional control (**A**), colostomy-free survival (**B**), and overall survival (**C**) of patients treated with either 5-FU or capecitabine-based chemoradiotherapy. In **B**, pretreatment colostomies that were reversed during follow-up are not shown.

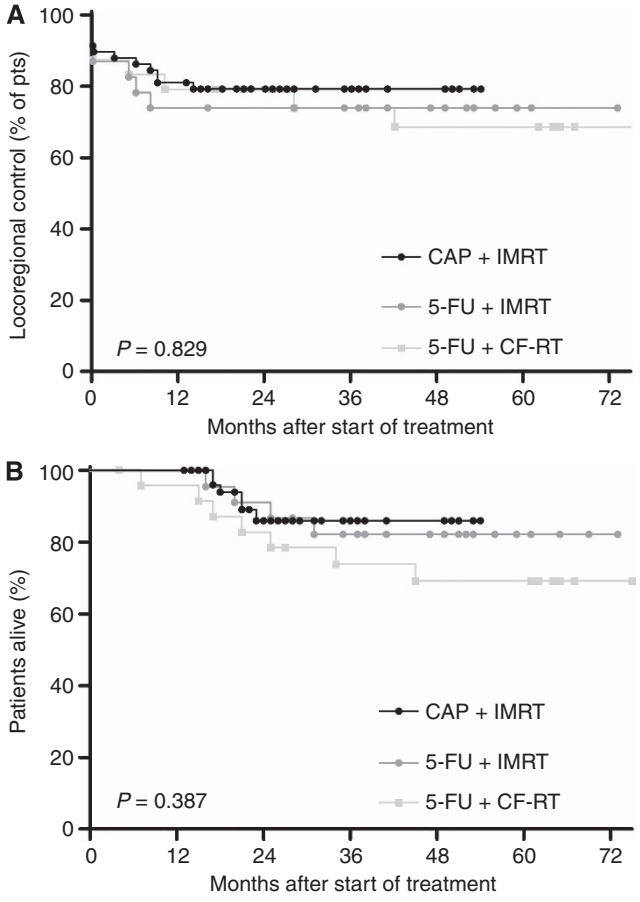

Figure 2. Outcome of subgroups of patients according to type of chemotherapy and radiation technique. The figures show the locoregional control (**A**) and overall survival (**B**) of patients treated with capecitabine/IMRT, 5-FU/IMRT, and 5-FU/CF-RT.

initially excluding six patients from treatment with capecitabine. However, the proportion of these patients that was disease-free and alive at last follow-up (83%) was comparable to the overall population. The type of radiotherapy that patients received may also have affected outcome. Superior outcome with IMRT has been claimed, due to the lack of a treatment break and shorter overall treatment time (Pepek *et al*, 2010; Bazan *et al*, 2011). However, inferior outcomes with CF-RT have mainly been demonstrated with longer treatment gaps ($\geqslant$5 weeks), or when the break is introduced early in the course of treatment (Weber *et al*, 2001; Glynne-Jones *et al*, 2011). In our study, the average treatment break with 5-FU + CF-RT was 3 weeks (± 4.5 days) and introduced after 45 Gy. We chose a higher total dose to the LNs with IMRT to compensate for the difference in biological effect of the daily dose of 1.5 Gy as compared with 1.8 Gy, using the linear-quadratic formalism of iso-effective dose calculations for late responding tissues with a α-β ratio of 3 Gy (Barendsen, 1982). Inherently, this results in a somewhat higher biological dose to the LNs as compared with CF-RT. We investigated, in a separate analysis, outcomes of patients receiving CF-RT + 5-FU ($n = 24$) *vs* IMRT + 5-FU ($n = 23$) and found highly similar results with regard to LRC and other parameters, not suggestive of confounding by radiation technique. Lastly, the duration of follow-up of patients treated with capecitabine was relatively short. It has, however, been demonstrated that the cCR rate is a good predictor for disease-free survival (Deniaud-Alexandre *et al*, 2003) and, that most locoregional failures occur within the first 2 years after treatment (UKCCCR Anal Cancer Trial Working Party, 1996; Bartelink *et al*, 1997).

CONCLUSION

In this retrospective analysis, we show for the first time that AC patients treated with capecitabine fare equally well as patients treated with 5-FU in terms of cCR rate, LRC and OS. Despite the above-mentioned limitations, we believe that the conclusions drawn from this study with regard to the primary end points

Table 4. Associations of genetic polymorphisms in *GSTT1*, *GSTM1*, *GSTP1* and *TYMS* with outcome and toxicity

	Clinical response			Overall toxicity		
Polymorphism	**PR**	**CR**	**P-value[a]**	**Grade 0–2**	**Grade 3–4**	**P-value[a]**
***GSTT1* (deletion)**						
Not NULL	7 (9%)	68 (91%)	0.686	55 (72%)	21 (28%)	0.065
NULL	3 (14%)	18 (86%)		10 (48%)	11 (52%)	
***GSTM1* (deletion)**						
Not NULL	4 (11%)	34 (89%)	1.000	26 (68%)	12 (32%)	0.827
NULL	6 (10%)	52 (90%)		38 (66%)	20 (35%)	
***GSTP1* 313A>G**						
AA	5 (12%)	38 (88%)	0.749	33 (77%)	10 (23%)	0.124
AG or GG	5 (9%)	48 (91%)		32 (60%)	21 (40%)	
***TYMS* 3′-UTR 6-bp ins/del**						
Ins/Ins	6 (15%)	33 (85%)	0.307	25 (64%)	14 (36%)	0.657
Ins/Del or Del/Del	4 (7%)	53 (93%)		40 (70%)	17 (30%)	
***TYMS* 5′-UTR VNTR[b]**						
High expressor	4 (12%)	30 (88%)	0.739	28 (82%)	6 (18%)	0.025
Low expressor	6 (10%)	56 (90%)		37 (60%)	25 (40%)	

Abbreviations: CR = complete response; *GSTM1* = glutathione S-transferase mu; *GSTP1* = glutathione S-transferase pi; *GSTT1* = glutathione S-transferase theta; *TYMS* = thymidylate synthase; PR = partial response; VNTR = variable number of 28-bp tandem repeats.
[a]Fisher's exact test (two-sided).
[b]Low *TYMS* expression genotypes are (*2/*2, *2/*3C and *3C/*3C) and high *TYMS* expression genotypes (*2/*3G, *3C/*3G or *3G/*3G).

Table 5. Associations of genetic polymorphisms in *GSTT1*, *GSTM1*, *GSTT1*, and *TYMS* with individual types of toxicity

	Dermatological toxicity			Haematological toxicity			Gastrointestinal toxicity			Genitourinary toxicity		
	Grade 0–2	**Grade 3–4**	***P*-value[a]**	**Grade 0–2**	**Grade 3–4**	***P*-value[a]**	**Grade 0–2**	**Grade 3–4**	***P*-value[a]**	**Grade 0–2**	**Grade 3-4**	***P*-value[a]**
GSTT1												
Not NULL	61 (81%)	14 (19%)	0.040	72 (96%)	3 (4%)	0.300	73 (97%)	2 (3%)	0.527	73 (97%)	2 (3%)	0.527
NULL	12 (57%)	9 (43%)		19 (90%)	2 (10%)		20 (95%)	1 (5%)		20 (95%)	1 (5%)	
GSTM1												
Not NULL	29 (76%)	9 (24%)	1.000	36 (95%)	2 (5%)	1.000	36 (95%)	2 (5%)	0.560	38 (100%)	0 (0%)	0.275
NULL	44 (76%)	14 (24%)		55 (95%)	3 (5%)		57 (98%)	1 (2%)		55 (95%)	3 (5%)	
GSTP1												
AA	36 (84%)	7 (16%)	0.223	42 (98%)	1 (2%)	0.376	42 (98%)	1 (2%)	1.000	42 (98%)	1 (2%)	1.000
AG or GG	38 (72%)	15 (28%)		49 (92%)	4 (8%)		51 (96%)	2 (4%)		51 (96%)	2 (4%)	
***TYMS* 3′-UTR 6-bp ins/del**												
Ins/Ins	29 (74%)	10 (26%)	0.628	36 (92%)	3 (8%)	0.393	38 (97%)	1 (3%)	1.000	37 (95%)	2 (5%)	0.564
Ins/Del or Del/Del	45 (79%)	12 (21%)		55 (96%)	2 (4%)		55 (96%)	2 (4%)		56 (98%)	1 (2%)	
***TYMS* 5′-UTR VNTR[b]**												
High expressor	30 (88%)	4 (12%)	0.075	32 (94%)	2 (6%)	1.000	34 (100%)	0 (0%)	0.550	34 (100%)	0 (0%)	0.550
Low expressor	44 (71%)	18 (29%)		59 (95%)	3 (5%)		59 (95%)	3 (5%)		59 (95%)	3 (5%)	

Abbreviations: *GSTM1* = glutathione S-transferase mu; *GSTP1* = glutathione S-transferase pi; *GSTT1* = glutathione S-transferase theta; *TYMS* = thymidylate synthase; VNTR = variable number of 28-bp tandem repeats.
[a]Fisher's exact test (two-sided).
[b]Low *TYMS* expression genotypes are (*2/*2, *2/*3C and *3C/*3C) and high *TYMS* expression genotypes (*2/*3G, *3C/*3G or *3G/*3G).

are valid. Our population reflects the treatment of AC in daily clinical practice, and we conclude that capecitabine 825 mg m^{-2} b.i.d. on radiation days can be used instead of continuous intravenous 5-FU in combination with MMC and IMRT in the treatment of locally advanced AC.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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