Docetaxel, cisplatin, and fluorouracil chemotherapy for metastatic or unresectable locally recurrent anal squamous cell carcinoma (Epitopes-HPV02): a multicentre, single-arm, phase 2 study

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Summary

Background The incidence of anal squamous cell carcinoma has been increasing markedly in the past few decades. Currently, there is no validated treatment for advanced-stage anal squamous cell carcinoma. Therefore, we aimed to validate the clinical activity and safety of docetaxel, cisplatin, and fluorouracil (DCF) chemotherapy in patients with metastatic or unresectable locally recurrent anal squamous cell carcinoma.

Methods We did a multicentre, single-arm, phase 2 study. We recruited patients from 25 academic hospitals, cancer research centres, and community hospitals in France who were aged 18 years or older with histologically confirmed anal squamous cell carcinoma, with metastatic disease or with unresectable local recurrence; an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1; and with at least one evaluable lesion according to the Response Evaluation Criteria in Solid Tumors (version 1.1). Chemotherapy-naive patients received either six cycles of standard DCF (75 mg/m² docetaxel and 75 mg/m² cisplatin on day 1 and 750 mg/m² per day of fluorouracil for 5 days, every 3 weeks) or eight cycles of modified DCF (40 mg/m² docetaxel and 40 mg/m² cisplatin on day 1 and 1200 mg/m² per day of fluorouracil for 2 days, every 2 weeks), which were administered intravenously. The choice between the standard versus modified regimens was recommended based on, but not limited to, age (≤75 years *vs* >75 years) and ECOG performance status (0 *vs* 1). The primary endpoint was investigator-assessed progression-free survival at 12 months from the first DCF cycle; for the primary endpoint to be met, at least 11 (17%) of 66 enrolled patients had to be alive without disease progression at 12 months. Efficacy and safety analyses were done in a modified intention-to-treat population, defined as all patients who were evaluable for progression at 12 months who received at least one cycle of DCF. This trial is registered at ClinicalTrials.gov, number NCT02402842, and the final results are presented here.

Findings Between Sept 17, 2014, and Dec 7, 2016, we enrolled 69 patients. Of these patients, three did not receive DCF. Of the 66 patients who received treatment, 36 received the standard DCF regimen and 30 received modified DCF. The primary endpoint was met: 31 (47%) of 66 patients were alive and progression free at 12 months. 22 (61%) of 36 patients who received the standard DCF regimen and 18 (60%) of 30 patients who received the modified DCF regimen had disease progression at data cutoff. 46 (70%) of 66 patients had at least one grade 3–4 adverse event (30 [83%] of 36 in the standard DCF regimen and 16 [53%] of 30 in the modified DCF regimen). The most common grade 3–4 adverse events were neutropenia (15 [23%]; eight [22%] for standard DCF *vs* seven [23%] for modified DCF), diarrhoea (12 [18%]; nine [25%] *vs* three [10%]), asthenia (ten [15%]; eight [22%] *vs* two [7%]), anaemia (ten [15%]; six [17%] *vs* four [13%]), lymphopenia (eight [12%]; three [8%] *vs* five [17%]), mucositis (seven [11%]; seven [19%] *vs* none), and vomiting (seven [11%]; five [14%] *vs* two [7%]). No grade 4 non-haematological adverse events and febrile neutropenia were observed with modified DCF, whereas three (8%) grade 4 non-haematological adverse events and five (14%) cases of febrile neutropenia were reported with standard DCF. 97 serious adverse events were reported (69 in patients who received the standard DCF regimen [61 drug-related] and 28 in those given the modified DCF regimen [14 drug-related]). No treatment-related deaths were recorded.

Interpretation Compared with standard DCF, modified DCF provided long-lasting response with good tolerability in patients with metastatic or unresectable locally recurrent anal squamous cell carcinoma with ECOG performance status of 0–1 in the first-line setting, and therefore could be considered as a new standard of care for these patients. Regarding the elevated risk of high-grade and serious adverse events and febrile neutropenia, standard DCF cannot be recommended in this situation.

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Introduction

Anal squamous cell carcinoma is still considered a rare disease, accounting for less than 3% of all gastrointestinal malignancies worldwide.[1] However, the incidence of this cancer has been increasing substantially in the past few decades and it is estimated that it will continue to increase in the foreseeable future, reflecting its association with human papillomavirus (HPV) infection.[2] HPV-related oncoproteins (ie, E6 and E7) are expressed in more than 90% of patients with anal squamous cell carcinoma.[3] About 15% of these patients are diagnosed at the metastatic stage. Moreover, local or metastatic recurrences occur in more than 20% of these patients treated initially by chemoradiotherapy.[4,5] Salvage surgery might be proposed to patients in case of resectable local progression. When surgery is not possible, the current recommended treatment involves systemic chemotherapy. However, at present, evidence-based data defining the appropriate chemotherapy regimen are inadequate.[6] On the basis of a retrospective analysis of 19 patients, the combination of cisplatin plus fluorouracil (CF) is recommended for advanced anal squamous cell carcinoma.[4,7] Nevertheless, only one (5%) of 19 patients had a complete remission and most patients had disease progression within 12 months after treatment. Although three (16%) of 19 patients who received CF achieved a durable remission, all three were treated with complementary surgery or radiotherapy. More recently, larger retrospective studies showed similar results, proposing CF chemotherapy as a suitable front-line treatment option in the absence of prospective data.[8,9] Although between 34% and 55% of patients achieved an objective response, no complete responses have been observed with CF chemotherapy in patients with anal squamous cell carcinoma, and long-term survival was limited to patients treated with complementary surgery

Research in context

Evidence before this study

We searched PubMed for clinical trials published up to March 25, 2018, using the search terms "anus neoplasms (MeSH term)" OR "anal cancer" and "advanced" OR "metastatic". We found three prospective phase 2 studies of first-line metastatic or non-resectable locally advanced anal squamous cell carcinoma. One study with mitomycin-C, doxorubicin, cisplatin, and bleomycin-CCNU (MAC-BC) in 20 patients with advanced anal squamous cell carcinoma; one with bleomycin, vincristine, and high-dose methotrexate (BOM) in 15 patients; and one with a triple combination of paclitaxel, carboplatin, and fluorouracil (PCaF) in seven patients with anal squamous cell carcinoma among 60 patients with advanced squamous carcinomas of various primary sites. No complete response was observed with MAC-BC and BOM, and two (29%) complete responses were reported with PCaF. At the time of the study design, there was no validated treatment for advanced-stage anal squamous cell carcinoma. Advanced diseases were only treated in a palliative setting. The association of cisplatin and fluorouracil (CF) has long been recommended on the basis of retrospective data. However, pooling five retrospective studies of CF chemotherapy in patients with advanced anal squamous cell carcinoma, only one complete response was reported in 92 patients. It has been previously proposed that a loss of normal p53 function confers sensitisation to taxane chemotherapy by increasing G2/M cell cycle arrest and apoptosis. Since the association between anal squamous cell carcinoma and human papillomavirus (HPV) infection is especially strong, and the E6 oncoprotein encoded by HPV-16 and HPV-18 induces the degradation of p53, we postulated that anal squamous cell carcinoma might be sensitive to taxane-containing chemotherapies, such as docetaxel. Additionally, docetaxel has been previously shown to increase endoplasmic reticulum stress and to induce immunogenic cell death of cancer cells. Considering the poor outcomes recorded with CF chemotherapy, we decided to treat patients with metastatic and unresectable local recurrent anal squamous cell carcinoma using a docetaxel, cisplatin, and fluorouracil (DCF) regimen. Unexpectedly, four (50%) of the first eight consecutive patients achieved a complete response, including three pathological complete responses. Consequently, we did a prospective trial to validate the clinical potential of DCF chemotherapy in patients with metastatic or unresectable locally recurrent anal squamous cell carcinoma.

Added value of this study

In this phase 2 trial of patients with first-line advanced anal squamous cell carcinoma, DCF was highly effective. A high proportion of complete responses and long-term remissions was achieved. We report that modified DCF offers a better safety profile than standard DCF in this population. The monitoring of antigen-specific T lymphocytes showed that DCF chemotherapy is immunogenic in this disease. The presence of anti-telomerase-specific T-helper-1 lymphocytes was correlated to the prognosis. These results raise the hypothesis that telomerase might be an attractive antigen in anal squamous cell carcinoma.

Implications of all the available evidence

On the basis of the results of this trial, modified DCF could be considered as standard of care for patients with metastatic or unresectable locally recurrent anal squamous cell carcinoma with an Eastern Cooperative Oncology Group performance status of 0 or 1. Taking into account that DCF is able to induce adaptive immune responses, modified DCF could be evaluated as a backbone chemotherapy for immunotherapy combinations in anal squamous cell carcinoma.

or radiotherapy. Thus, outcomes for patients with non-resectable or metastatic disease remain poor, with 5-year overall survival below 20% for patients with stage IV disease.[10] There is an unmet need for effective salvage systemic therapies for patients with anal squamous cell carcinoma.

Docetaxel is a potent microtubule-stabilising agent with anti-tumour activity that leads to mitosis arrest and cell death. It has been previously proposed that a loss of normal p53 function confers sensitisation to taxane chemotherapy by increasing G2/M cell arrest and apoptosis.[11] Because the association between anal squamous cell carcinoma and HPV infection is substantial and the E6 oncoprotein encoded by HPV-16 and HPV-18 induces the degradation of p53, we previously hypothesised that anal squamous cell carcinoma might be sensitive to taxane-containing chemotherapies, such as docetaxel.[3,12] Additionally, docetaxel has been previously shown to increase endoplasmic reticulum stress and to induce immunogenic cell death of cancer cells.[13] The improvement of overall survival without asubstantial effect on toxicity through the addition of docetaxel to cisplatin and fluorouracil (DCF) chemotherapy has already been shown in advanced squamous cell carcinoma of the head and neck.[14]

Results from a first cohort of eight consecutive patients with anal squamous cell carcinoma treated with DCF were previously published as a retrospective study.[15] Several observations derived from this retrospective analysis form the basis of the rationale for the current Epitopes-HPV02 trial. First, four (50%) of these eight patients achieved a complete response. The magnitude of DCF activity was histologically confirmed, since pathological complete responses were observed in three (75%) of these four patients who underwent surgery for metastatic disease. Additionally, DCF chemotherapy was active in patients who had recurrence after radiotherapy, suggesting that this triplet chemotherapy regimen might be a potential salvage therapy for locoregional recurrence. Lastly, complete remissions in four (50%) of eight patients reported in this preliminary study were durable because all these patients were disease-free at the time of last follow-up (about 8 years). On the basis of these encouraging results, and considering the poor outcomes achieved with CF chemotherapy in patients with metastatic and unresectable local recurrent anal squamous cell carcinoma, we decided to prospectively validate DCF chemotherapy in this setting.[15,16]

Methods

Study design and participants

We did a multicentre, single-arm, phase 2 study, which was conducted by the Besançon University Hospital (Besançon, France) and Clinical Investigational Centre (INSERM CIC 1431, Besançon, France) and supported by the GERCOR and FFCD collaborative oncological groups. Patients were recruited from 25 academic hospitals, cancer research centres, and community hospitals in France (appendix p 18).

See Online for appendix

We included patients aged 18 years or older with histologically confirmed anal squamous cell carcinoma, metastatic disease (regardless of resectability), or with unresectable local recurrence (regardless of timeframe following local treatment) after chemoradiotherapy; an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1; with at least one evaluable lesion according to the Response Evaluation Criteria in Solid Tumors (RECIST; version 1.1); and were eligible for DCF chemotherapy (ie, no exclusion criteria related to DCF chemotherapy). Patients previously exposed to cisplatin or fluorouracil, or both (CF chemotherapy), were eligible if the chemotherapy regimen was administered as concomitant treatment of radiotherapy for local or locally advanced disease. Additionally, patients had to have adequate organ function (an absolute neutrophil count ≥1500 cells per mm³, platelet count ≥100 000 cells per mm³, creatinine clearance [according to Cockcroft formula] ≥60 mL/min, aspartate aminotransferase and alanine aminotransferase ≤2·5×upper limit of normal [ULN; or ≤5×ULN in the case of known liver metastases], and total bilirubin ≤2·5×ULN). HIV-positive patients were allowed to participate if their CD4 count was 400 cells or more per mm³.

We excluded patients who were previously exposed to chemotherapy for metastatic disease, or to paclitaxel, docetaxel, or vinorelbine. Patients receiving treatment with a CYP3A4 inhibitor were excluded unless replacement was possible before inclusion. Additionally, we excluded patients if they had an uncontrolled cardiovascular or pulmonary disease, neuropathy, or hearing impairment, and women who were pregnant or of childbearing potential.

This study was reviewed and approved by the independent Est-II French Committee for Protection of Persons on June 6, 2014, and by the French Health Products Safety Agency on July 15, 2014, and was done in accordance to the Declaration of Helsinki. All patients provided written informed consent before study enrolment.[16]

Procedures

Chemotherapy-naive patients received either six cycles of a standard DCF regimen (75 mg/m² docetaxel and 75 mg/m² cisplatin on day 1 and 750 mg/m² per day of fluorouracil for 5 days, every 3 weeks) or eight cycles of a modified DCF regimen (40 mg/m² docetaxel and 40 mg/m² cisplatin on day 1 and 1200 mg/m² per day of fluorouracil for 2 days, every 2 weeks), which were administered intravenously. The choice between standard or modified DCF was based on the participants' clinical status (ie, performance status and age) or investigator choice. Standard DCF was recommended, although not mandatory, for patients aged 75 years or younger and with an ECOG performance status of 0, and

modified DCF was recommended for those older than 75 years or with an ECOG performance status of 1.

Granulocyte colony-stimulating factor (G-CSF; lenograstim or filgrastim) was administered subcutaneously at the recommended dose of 5 µg/kg per day, for 5 days (for modified DCF) to 7 days (for standard DCF), as primary prophylaxis of febrile neutropenia for both regimens, as well as to maintain the dose intensity planned. Adverse events were assessed by the investigators before each DCF cycle and graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (version 4.03). DCF was continued at the full dose in case of treatment-related grade 1 adverse events or grade 1–2 neutropenia. The chemotherapy was withheld in case of grade 2 or grade 3 neutropenia until recovery. In case of delay or serious adverse events, the dose of the chemotherapy drug involved in the toxicity was reduced by 25%. The appendix (p 14) provides further details on the dose reduction and treatment interruption criteria.

At the end of the planned DCF cycles, complementary local treatments such as surgery of metastases or radiotherapy were allowed at the discretion of each local institutional multidisciplinary board. CT scans were done at baseline, after the third and sixth cycles of standard DCF or after the fourth and eighth cycles of modified DCF, and then every 3 months until disease progression or death. Responses to treatment were assessed by the site investigator and by a central reviewer who were masked to study data, according to RECIST. CT scans were anonymised in each site and then centralised by the Department of Clinical Research and Innovation of University Hospital of Besançon (Besançon, France). A radiologist who was masked to study data did the analysis of the CT scans in accordance with RECIST. A PET scan was mandatory before and at the end of treatment (defined as 28 days after the last cycle of DCF). No PET scans were required at disease progression.

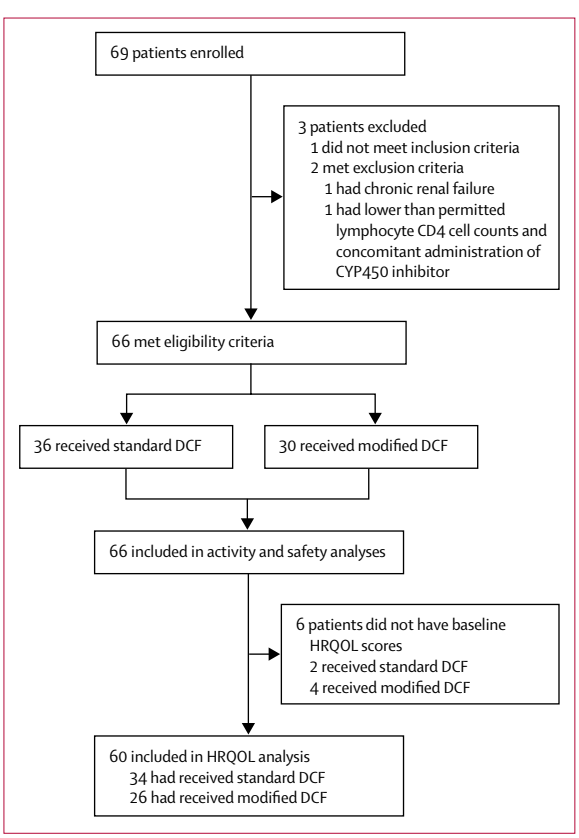

Figure 1: **Trial profile**
DCF=docetaxel, cisplatin, and fluorouracil. HRQOL=health-related quality of life.

Health-related quality of life (HRQOL) was assessed using the European Organisation for Research and Treatment of Cancer (EORTC) QLQ-C30 questionnaire at inclusion, every two cycles of chemotherapy, and after treatment and at each follow-up visit. Blood samples for immuno-monitoring were taken before DCF administration and at first follow-up visit after the last DCF cycle. After the planned DCF cycles, patients were followed up every 3 months for up to 3 years from enrolment or to death. A CT scan, blood analysis, clinical examination, and HRQOL assessment were done at each follow-up visit. Criteria for removal from the study were withdrawal of consent or physician's decision.

We characterised HPV genotype using the Inno-LiPA assay (Innogenetics NV, Gent, Belgium). The viral load was not assessed. We assessed antigen-specific T-cell responses in isolated peripheral blood mononuclear cells at baseline and 1 month after the last DCF administration.[17] Peripheral blood mononuclear cells, which were isolated by the Ficoll-Hypaque technique, were exposed to E6 and E7 HPV-oncoprotein-derived peptides to analyse the presence of specific T cells. Peripheral blood mononuclear cells were also exposed to human telomerase (hTERT)-derived peptides, since it was previously reported that E6 oncoprotein transactivates hTERT.[18] Antigen-specific CD4 T-cell responses were monitored by the Enzyme-Linked ImmunoSpot assay (ELISPOT), using promiscuous peptides that were designed to allow the assessment of DCF chemotherapy on hTERT-specific CD4 T-helper-1 (Th1) immunity.[17] The appendix (p 1) provides further details about methods of cell isolation and secreted cytokine detection.

Outcomes

The primary endpoint was investigator-assessed progression-free survival (defined as the number of patients alive and without documented disease progression as per RECIST) at 12 months from the first DCF administration in all evaluable patients. Secondary endpoints were progression-free survival (defined as the time between the treatment initiation date and the date of the first progression [local, regional, metastatic, or secondary cancer] or the last follow-up date for patients who were alive without progression), overall survival (defined as the time between the treatment initiation

date and date of death from any cause), the proportion of patients who achieved an objective response (partial response or complete response, according to RECIST), safety, HRQOL, and tissue and immune biomarkers, including HPV and telomerase-specific T cell monitoring before and after DCF treatment. All secondary endpoints were analysed in an exploratory way, whereby the number of patients needed was calculated according to the primary endpoint.

Statistical analysis

We designed this study with Simon's optimal two-stage method, which allows stopping early for futility at the first stage (ie, insufficient activity of the DCF regimen), based on the results of an interim analysis. A 12-month progression-free survival of 25% was expected (H_1: alternative hypothesis), whereas a 12-month progression-free survival of 10% was considered as uninteresting (H_0: null hypothesis). With a statistical power of 90% and a one-sided type 1 error of 0·05, 66 evaluable patients were required for the final analysis of progression-free survival (at the data cutoff date of Nov 24, 2017), of which at least 11 patients (17%) were required to be alive and progression free at 12 months. For the first stage (ie, interim analysis), 21 patients had to be enrolled, of whom at least three patients had to be alive and progression-free at 12 months to allow the inclusion of 45 additional patients for the final analysis. For the interim analysis (cutoff date Sept 5, 2016), activity and safety data were extracted from electronic case report forms by independent departments, and subsequently, the complete interim database became available to the Department of Clinical Research and Innovation of University Hospital of Besançon.[16]

All patients who received at least one cycle of DCF were included in the activity and safety analyses (modified intention-to-treat). For the HRQOL analysis, only those patients with at least one baseline HRQOL score available were included.

Medians and IQRs were provided for the description of continuous variables and frequency and percentages were provided for the description of categorical variables. The median progression-free survival and overall survival and the proportion of patients who met these endpoints at specific timepoints were estimated by the Kaplan-Meier method. 95% CIs were determined with the log-log transformation. Median follow-up was calculated by the reverse Kaplan-Meier method.

Cox proportional-hazard models were used to estimate hazard ratios (HRs) and their 95% CIs for factors associated with progression-free survival. The association of baseline parameters with progression-free survival was evaluated in a prespecified exploratory analysis using the univariate Cox model.

For HRQOL analysis, the EORTC QLQ-C30 was used. After the generation of the scores following the EORTC recommendations, the results were described at baseline by type of regimen (standard or modified DCF) using median (IQR) and mean (SD). The longitudinal analysis of HRQOL was done according to the time until definitive deterioration (TUDD) approach,[19] which evaluates the time until definitive HRQOL deterioration. The TUDD

	Overall study population treated with DCF (n=66)	Standard DCF group (n=36)	Modified DCF group (n=30)
Median age, years	60·1 (53·6–65·8)	59·8 (53·5–64·1)	63·1 (54·3–67·4)
Age group, years			
<65	47 (71%)	28 (78%)	19 (63%)
≥65	19 (29%)	8 (22%)	11 (37%)
Sex			
Women	54 (82%)	28 (78%)	26 (87%)
Men	12 (18%)	8 (22%)	4 (13%)
Advanced disease diagnosis			
Synchronous	16 (24%)	8 (22%)	8 (27%)
Metachronous	44 (67%)	25 (69%)	19 (63%)
Non-resectable locally advanced	6 (9%)	3 (8%)	3 (10%)
Previous chemoradiotherapy	45 (68%)	24 (67%)	21 (70%)
Mitomycin and fluorouracil	28 (64%)	15 (63%)	13 (62%)
Mitomycin and capecitabine	6 (14%)	2 (8%)	4 (19%)
Mitomycin	1 (2%)	0	1 (5%)
Capecitabine	1 (2%)	1 (4%)	0
Cisplatin and fluorouracil	6 (14%)	4 (17%)	2 (10%)
Cisplatin, mitomycin, and fluorouracil	1 (2%)	1 (4%)	0
Cisplatin, mitomycin, fluorouracil, and capecitabine	1 (2%)	0	1 (5%)
Missing	1	1	0
Median time to advanced disease, months	11·08 (6·67–26·33)	10·83 (4·80–33·40)	15·32 (6·94–24·03)
Number of involved sites			
1	22 (33%)	13 (36%)	9 (30%)
2	18 (27%)	9 (25%)	9 (30%)
3	12 (18%)	8 (22%)	4 (13%)
≥4	14 (21%)	6 (17%)	8 (27%)
Involved sites			
Locoregional	41 (62%)	22 (61%)	19 (63%)
Distant lymph nodes	32 (48%)	14 (39%)	18 (60%)
Liver	40 (61%)	24 (67%)	16 (53%)
Lung	24 (36%)	11 (31%)	13 (43%)
Bone	7 (11%)	3 (8%)	4 (13%)
Peritoneal	3 (5%)	3 (8%)	0
Skin	3 (5%)	1 (3%)	2 (7%)
Other	8 (12%)	5 (14%)	3 (10%)
HIV status			
Positive	2 (3%)	2 (6%)	0
Negative	64 (97%)	34 (94%)	30 (100%)
ECOG performance status			
0	41 (62%)	25 (69%)	16 (53%)
1	25 (38%)	11 (31%)	14 (47%)
HPV positive			
Yes	55 (95%)	29 (97%)	26 (93%)
No	3 (5%)	1 (3%)	2 (7%)
Missing*	8	6	2

(Table 1 continues on next page)

	Overall study population treated with DCF (n=66)	Standard DCF group (n=36)	Modified DCF group (n=30)
(Continued from previous page)			
HPV type			
16	51 (93%)	28 (97%)	23 (88%)
33	1 (2%)	0	1 (4%)
Coinfection	3 (5%)	1 (3%)	2 (8%)
Missing	0	0	0
p16 positive			
Yes	56 (97%)	31 (97%)	25 (96%)
No	2 (3%)	1 (3%)	1 (4%)
Missing*	8	4	4
p53 positive			
Yes	4 (7%)	2 (6%)	2 (8%)
No	53 (93%)	30 (94%)	23 (92%)
Missing*	9	4	5

Data are median (IQR), n (%), or n. DCF=docetaxel, cisplatin, and fluorouracil. ECOG=Eastern Cooperative Oncology Group. HPV=human papillomavirus. *Missing data were not included in the denominator for better comparison between groups with data.

Table 1: **Baseline patient characteristics at inclusion in the activity and safety population**

of the given HRQOL score was defined as the interval of time between the date of enrolment and the observation of the first deterioration of at least 10 points of the HRQOL score compared with the baseline score, without later improvement of at least 10 points as compared with the baseline score.[19] Patients with no clinically notable deterioration were censored at the time of the last HRQOL questionnaire available. Each HRQOL scale of the QLQ-C30 was analysed separately.

We did all analyses using SAS (version 9.4) and the QoLR package in R (version 3.4.0).[20] This trial is registered with ClinicalTrials.gov, number NCT02402842.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Sept 17, 2014, and Dec 7, 2016, 69 patients with advanced anal squamous cell carcinoma were enrolled, of whom 66 (96%) were eligible for the activity and safety analyses (figure 1). Table 1 shows the baseline demographic characteristics; most patients were women, and most had an ECOG performance status of 0. Overall, 36 (55%) of 66 patients received standard DCF and 30 (45%) received modified DCF.

As per protocol, the interim analysis (cutoff date Sept 5, 2016) was done when the first 21 evaluable patients had completed 12 months of treatment with both DCF regimens, and showed that ten (48%) patients were alive and progression free. Based on these results, the trial continued.

The database was locked for the final analysis on Nov 24, 2017, with a median follow-up for all patients of 19·8 months (IQR 12·1–22·7). In this final analysis, 31 (47%) of 66 patients were alive and disease progression-free at 12 months. Considering the prespecified threshold of 11 (17%) patients being alive without disease progression at 12 months, the Epitopes-HPV02 trial met its primary endpoint.

57 (86%) of 66 patients achieved an objective response as per the investigator's assessment (including 29 [44%] with a complete response), whereas the blinded centralised analysis reported that 59 (89%) patients had an objective response (including 30 [45%] with a complete response). 64 (97%) of 66 patients achieved disease control (ie, complete response, partial response, or stable disease) according to both the investigators' and centralised analyses. 15 (42%) of 36 patients treated with standard DCF chemotherapy had a complete response and 32 (89%) had an objective response (figure 2). 14 (47%) of 30 patients treated with modified DCF had a complete response and 25 (83%) had an objective response. Two patients presented with disease progression at first evaluation. Both patients were refractory to chemoradiotherapy and one underwent surgery of primary tumour before inclusion into this study.

22 (61%) of 36 patients in the standard DCF regimen and 18 (60%) of 30 patients in modified DCF regimen had disease progression at data cutoff. The median progression-free survival for the overall population was 11·0 months (95% CI 9·3–16·4); 10·7 months (95% CI 8·7–not reached) for the standard DCF regimen group and 11·0 months (6·8–16·4) for the modified DCF regimen group (figure 3A).

49 (74%) of 66 patients were still alive at the time of the final analysis. Overall survival at 12 months for the overall population was 83·1% (95% CI 71·5–90·3); 83·3% (95% CI 66·6–92·1) for the standard DCF regimen group and 82·7% (63·2–92·4) for the modified DCF regimen group (figure 3B). The median overall survival for the overall population was not reached (95% CI 25·2–not reached); not reached (95% CI 25·2–not reached) for the standard DCF regimen group and not reached (23·5–not reached) for the modified DCF regimen group. Two patients who were HIV positive were included in the trial. One patient had stable disease as their best response before progression at 11·5 months from the first DCF cycle, and the other patient achieved a complete response of all involved sites and was still disease-free at 24 months of follow-up (appendix p 2).

Results from the prespecified exploratory univariate Cox models are presented in the appendix (p 5). Age and number of sites involved were associated with progression-free survival in the univariate analysis and were considered for multivariate analysis (appendix p 5). No factor was

significantly associated with progression-free survival in the multivariate analysis.

The mean number of cycles of standard DCF administered was 5·7 (SD 1·1) and the median was 6·0 (IQR 6·0–6·0), whereas the mean number of modified DCF cycles administered was 7·5 (1·5) and the median was 8·0 (8·0–8·0). 27 (75%) of 36 patients who received the standard DCF regimen had at least one dose reduction compared with 15 (50%) of 30 patients given the modified DCF regimen. The percentage of scheduled dose actually delivered for docetaxel was 82%, cisplatin was 75%, and fluorouracil was 79% in the standard DCF regimen; and the dose percentage delivered for docetaxel was 90%, cisplatin was 89%, and fluorouracil was 90% in the modified DCF regimen. The median total cumulative doses for both the standard and modified DCF regimens are shown in the appendix (p 4).

46 (70%) of 66 patients (30 [83%] of 36 in the standard DCF regimen and 16 [53%] of 30 in the modified DCF regimen) had at least one grade 3–4 adverse event, of which the most common were neutropenia (15 [23%] of 66 patients), diarrhoea (12 [18%]), asthenia (ten [15%]), anaemia (ten [15%]), lymphopenia (eight [12%]), mucositis (seven [11%]), vomiting (seven [11%]), and febrile neutropenia (five [8%]; table 2).

In the overall population, 77 grade 3–4 adverse events (50 non-haematological adverse events) were observed in the standard DCF regimen and 33 (17 non-haematological adverse events) in the modified DCF regimen. All the grade 4 non-haematological adverse events (mucositis [n=1], acute renal failure [n=1], and heart failure [n=1]) and all four events of febrile neutropenia occurred in the standard DCF regimen. Ten (15%) of 66 patients discontinued DCF: four patients discontinued because of treatment-related adverse events (all four received standard DCF; these events were diarrhoea, mucositis, anaemia, and neutropenia), two because of patient's decision (n=1 for both standard and modified DCF), and four from investigator's decision (n=2 for both standard and modified DCF).

97 serious adverse events were reported during the whole period of systemic chemotherapy (69 in patients who received the standard DCF regimen and 28 in those given the modified DCF regimen; appendix p 3), of which 75 drug-related serious adverse events (61 in the standard DCF regimen *vs* 14 in the modified DCF regimen). The most frequent drug-related serious adverse events were anaemia (eight in standard DCF), diarrhoea (six in standard DCF *vs* one in modified DCF), fatigue (five *vs* one), nausea (four *vs* two), neutropenia (three with each regimen), vomiting (three with each regimen), and febrile bone marrow aplasia (four with standard DCF).

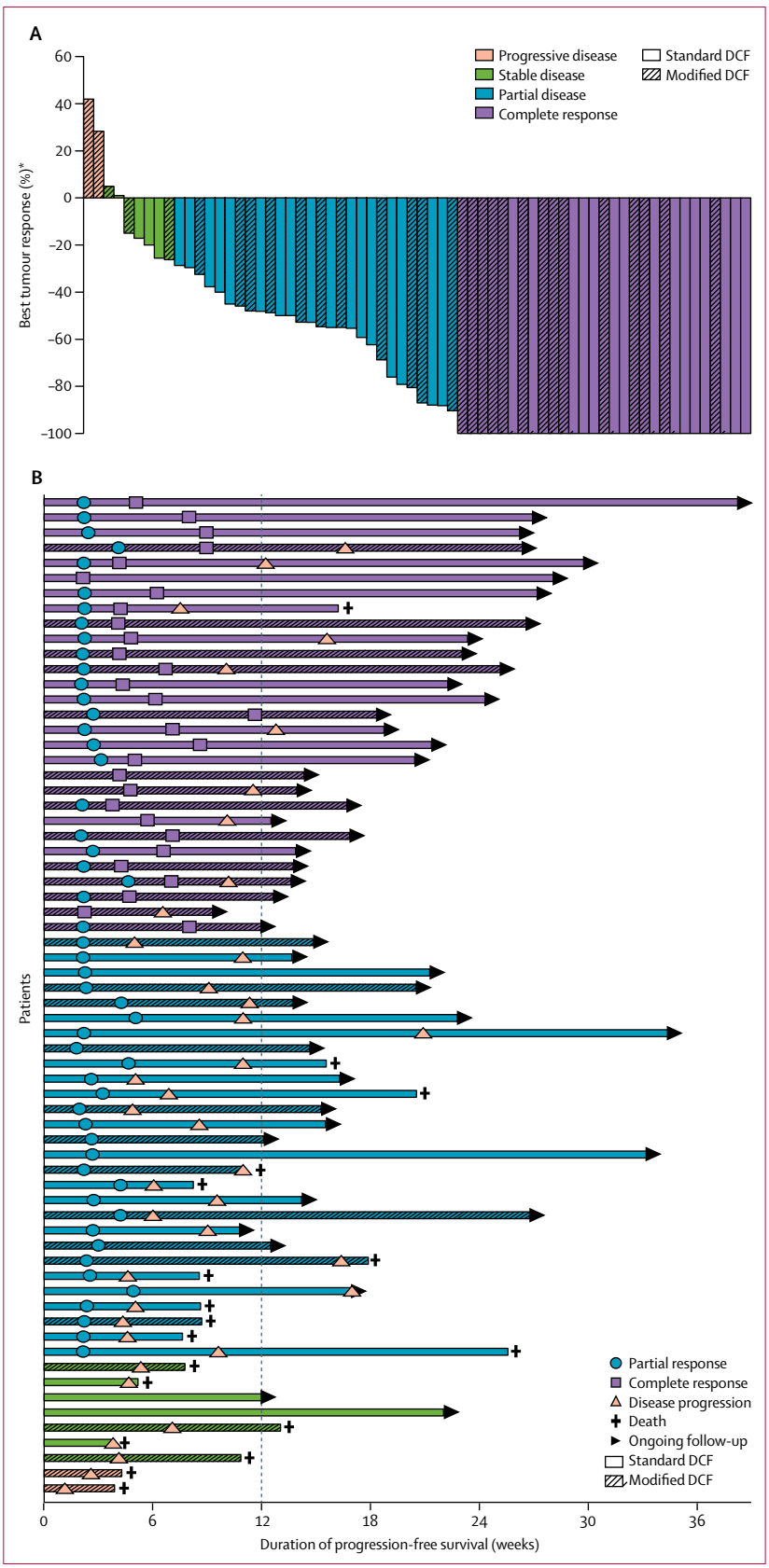

Figure 2: **Responses to treatment**
Best tumour response (A) and duration of progression-free survival (B) by type of response. Each bar represents a patient. DCF=docetaxel, cisplatin, and fluorouracil. *Change in tumour size from baseline.

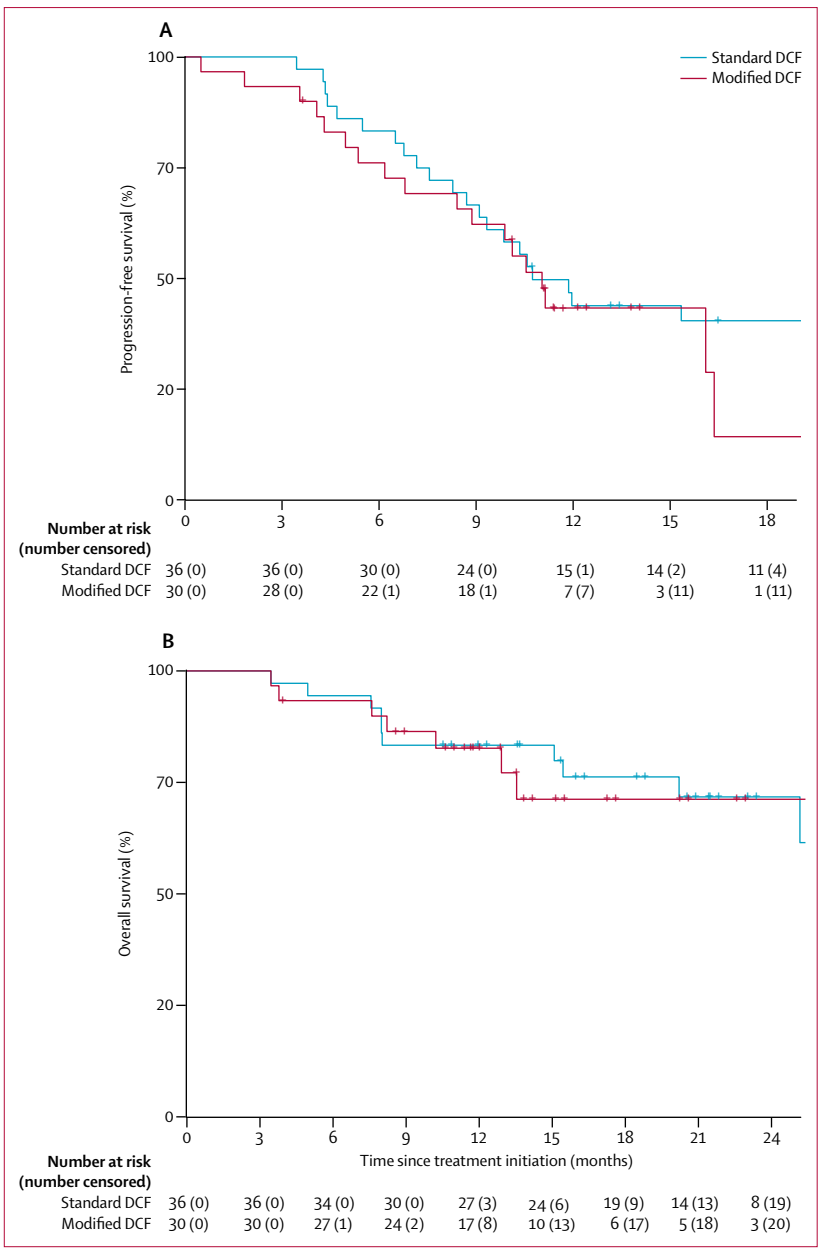

***Figure 3*: Progression-free survival (A) and overall survival (B)**
DCF=docetaxel, cisplatin, and fluorouracil.

17 (26%) of 66 patients (ten [28%] of 36 patients in the standard DCF regimen and seven [23%] of 30 patients in the modified DCF regimen) died as of the cutoff date in our study, with no treatment-related deaths reported. All reported deaths were due to disease progression, except for one patient who received the standard DCF regimen, who died because of an infection during second-line treatment.

DCF chemotherapy did not change absolute lymphocyte count in the study population, with the median absolute lymphocyte count remaining above 1100 cells per mm³ (median 1168 [IQR 800–1721], mean 1367·5 [SD 817·5]) during the treatment period (data not shown). Lymphocyte counts remained above 1200 cells per mm³ for the two HIV-positive patients during the entire treatment (appendix p 2).

Of the 66 eligible patients, 60 (91%) completed the HRQOL questionnaire at baseline and were included in the HRQOL analysis (figure 1). Similar HRQOL scores at baseline were found between both regimens except for constipation incidence, which was higher in patients given the modified DCF regimen than in those given the standard DCF regimen (data not shown). Results of all HRQOL dimensions are reported in the appendix (p 6). The TUDD in global health status or QOL score at 12 months was 40·7% (95% CI 25·6–55·3) for all patients (36·9% [18·7–55·2] for patients receiving the standard DCF regimen and 49·5% [25·1–70·0] for those receiving the modified DCF regimen). The TUDD of physical functioning at 12 months was 30·4% (95% CI 16·5–45·5%) for all patients (22·2% [95% CI 8·9–39·2] for standard DCF and 48·4% [21·1–71·3] for modified DCF). In a post-hoc analysis, the mean change over time of each HRQOL score by type of regimens is shown in the appendix (p 10) to highlight the longitudinal change of HRQOL level. We also performed a post-hoc exploratory analysis for each HRQOL dimension over time using a linear mixed model (appendix p 8).

Regarding the post-hoc efficacy analysis of DCF for anal squamous cell carcinoma relapses, overall 23 (35%) of 66 patients had pelvic relapses occurring in a previously irradiated area (with or without distant metastases). Of these patients, 19 (83%) had an objective response including ten (43%) who achieved complete responses (five with standard DCF and five with modified DCF). Nine (39%) of 23 patients were progression free at 12 months from the first cycle of the DCF regimen (five with standard DCF and four with modified DCF) and seven (30%) had no sign of recurrence at the time of analysis (four with standard DCF and three with modified DCF). Two (9%) patients had primary resistance to DCF with an early progression of their disease (two with modified DCF and none with standard DCF) in this post-hoc analysis. None of the 16 chemotherapy-naive patients with synchronous metastases had local or metastatic progression during DCF administration. All of these patients had achieved an objective response (eight with standard DCF and eight with modified DCF). Complete response was observed in nine (56%) of 16 patients (four with standard DCF and five with modified DCF), and ten (63%) patients were progression free at 12 months (five with standard DCF and five with modified DCF). Nine (56%) patients were still progression free at the time of final analysis (five with standard DCF and four with modified DCF).

14 (21%) of 66 patients underwent complementary treatment after DCF: eight (12%) had surgery of their metastatic disease, two (3%) received radiotherapy

	Overall study population treated with DCF (n=66)			Standard DCF group (n=36)			Modified DCF group (n=30)		
	Grade 1–2	Grade 3	Grade 4	Grade 1–2	Grade 3	Grade 4	Grade 1–2	Grade 3	Grade 4
Non-haematological adverse events									
Hypersensitivity	3 (5%)	0	0	3 (8%)	0	0	0	0	0
Anorexia	17 (26%)	2 (3%)	0	11 (31%)	2 (6%)	0	6 (20%)	0	0
Dysgeusia	11 (17%)	0	0	8 (22%)	0	0	3 (10%)	0	0
Nausea	45 (68%)	5 (8%)	0	28 (78%)	2 (6%)	0	17 (57%)	3 (10%)	0
Vomiting	20 (30%)	7 (11%)	0	16 (44%)	5 (14%)	0	4 (13%)	2 (7%)	0
Dehydration	1 (2%)	1 (2%)	0	0	1 (3%)	0	1 (3%)	0	0
Diarrhoea	35 (53%)	12 (18%)	0	19 (53%)	9 (25%)	0	16 (53%)	3 (10%)	0
Abdominal pain	12 (18%)	0	0	8 (22%)	0	0	4 (13%)	0	0
Constipation	11 (17%)	0	0	5 (14%)	0	0	6 (20%)	0	0
Mucositis	29 (44%)	6 (9%)	1 (2%)	18 (50%)	6 (17%)	1 (3%)	11 (37%)	0	0
Asthenia	46 (70%)	10 (15%)	0	25 (69%)	8 (22%)	0	21 (70%)	2 (7%)	0
Peripheral neuropathy	29 (44%)	4 (6%)	0	17 (47%)	2 (6%)	0	12 (40%)	2 (7%)	0
Alopecia	33 (50%)	..	..	19 (53%)	..	..	14 (47%)	..	..
Electrolytes									
Hypomagnesaemia	1 (2%)	0	0	0	0	0	1 (3%)	0	0
Hypokalaemia	3 (5%)	1 (2%)	0	3 (8%)	1 (3%)	0	0	0	0
Hyponatraemia	0	1 (2%)	0	0	0	0	0	1 (3%)	0
Thromboembolic event	1 (2%)	1 (2%)	0	1 (3%)	1 (3%)	0	0	0	0
Oedema in the limbs	10 (15%)	0	0	4 (11%)	0	0	6 (20%)	0	0
Gastrointestinal haemorrhage	0	2 (3%)	0	0	2 (6%)	0	0	0	0
ALT or AST elevation	2 (3%)	0	0	2 (6%)	0	0	0	0	0
Acute renal failure	2 (3%)	0	1 (2%)	1 (3%)	0	1 (3%)	1 (3%)	0	0
Heart failure	0	0	1 (2%)	0	0	1 (3%)	0	0	0
Oesophagitis	1 (2%)	1 (2%)	0	1 (3%)	1 (3%)	0	0	0	0
Hand-foot syndrome	8 (12%)	0	0	5 (14%)	0	0	3 (10%)	0	0
Infection	10 (15%)	3 (5%)	0	7 (19%)	2 (6%)	0	3 (10%)	1 (3%)	0
Weight loss	12 (18%)	1 (2%)	0	10 (28%)	0	0	2 (7%)	1 (3%)	0
Haematological adverse events									
Anaemia	34 (52%)	9 (14%)	1 (2%)	18 (50%)	6 (17%)	0	16 (53%)	3 (10%)	1 (3%)
Thrombocytopenia	12 (18%)	1 (2%)	1 (2%)	4 (11%)	1 (3%)	1 (3%)	8 (27%)	0	0
Lymphopenia	10 (15%)	8 (12%)	0	3 (8%)	3 (8%)	0	7 (23%)	5 (17%)	0
Leucopenia	4 (6%)	2 (3%)	1 (2%)	1 (3%)	2 (6%)	1 (3%)	3 (10%)	0	0
Neutropenia	11 (17%)	8 (12%)	7 (11%)	8 (22%)	5 (14%)	3 (8%)	3 (10%)	3 (10%)	4 (13%)
Febrile neutropenia	..	1 (2%)	4 (6%)	..	1 (3%)	4 (11%)	..	0	0

Data are n (%), for which a patient could have more than one adverse event. DCF=docetaxel, cisplatin, and fluorouracil. ALT=alanine aminotransferase. AST=aspartate aminotransferase.

Table 2: **Adverse events in the safety population**

(one stereotaxic radiotherapy of the liver metastases and one chemoradiotherapy of the primary tumour), and four (6%) had synchronous anal squamous cell carcinoma with distant metastases in one to three different sites (appendix p 12). Among these last four patients, three had a partial radiological response and one had a complete response after DCF treatment. All four patients underwent liver surgery (showing pathological complete responses in two patients) and chemoradiotherapy of their primary tumour site. Pathological complete response was reported in five (63%) of the eight patients who underwent surgery for their metastatic disease. None of these patients received radiotherapy or any complementary treatment between DCF and surgery. Six (43%) of the 14 patients who received surgical or chemoradiotherapy-based consolidation therapies presented with disease progression, and eight (57%) were still progression free at the time of the final analysis. No palliative radiotherapy was given in this trial.

In a post-hoc exploratory analysis, nine (64%) of 14 patients who received complementary treatment achieved progression-free survival at 12 months and median progression-free survival was not reached in this cohort.

Meanwhile, 22 (42%) of 52 patients who did not receive complementary treatment achieved 12-month progression-free survival; these patients had a median progression-free survival of 10·12 months (95% CI

7·56–15·34) and 20 (39%) of 52 patients had a radiological complete response.

There were 64 assessable patients for immunomonitoring; the blood sample at baseline was not done for two of 66 patients. At baseline, ten (16%) of 64 patients enrolled had detectable T-cell responses targeting HPV-16-E6 peptides. Interferon-γ production by HPV-16-E7-specific-T cells was detected in six (9%) of 64 patients. 17 (27%) of 64 patients had anti-hTERT CD4 Th1 lymphocytes in their peripheral blood. The frequency of HPV-related specific immune responses was increased after DCF treatment: of the 50 assessable patients, 17 (34%) had antigen-specific T cells recognising HPV-16-E6, eight (16%) recognised HPV-16-E7, and 16 (32%) recognised hTERT 1 month after the last DCF cycle. The median of interferon-γ ELISPOT ratio for E6 was 8·3 (IQR 2·3–3·0) after DCF therapy versus 5·0 (2·0–22·0) at baseline, for E7 was 7·6 (2·5–25·3) versus 2·9 (2·0–12·0), and for hTERT was 7·0 (2·1–54·0) versus 6·1 (2·3–34·6). Notably, HPV or hTERT measured at baseline did not significantly affect the prognosis of patients with anal squamous cell carcinoma: in a post-hoc exploratory analysis the median progression-free survival in patients without anti-HPV adaptive immunity was 11·5 months (95% CI 8·9–16·4) and with anti-HPV adaptive immunity was 10·4 months (95% CI 8·4–20·6; p=0·50). A median progression-free survival of 16·4 months (95% CI 10·6–not reached) was observed in patients responding to hTERT-derived peptides at baseline versus 10·4 months (8·7–15·35) in those without responses (p=0·059). Median progression-free survival was 20·6 months (95% CI 15·3–not reached) in responders and 10·1 months (8·3–11·5) in non-responders to major histocompatibility complex class II-restricted hTERT peptides after DCF treatment (p=0·04; appendix p 13). Moreover, only anti-hTERT Th1 CD4 T cell responses measured after DCF chemotherapy predicted the probability of progression-free survival at 12 months (ten [62·5%] of 16 patients in hTERT immune responders *vs* eight [23·5%] of 34 patients in non-responders; p=0·017).

Discussion

In this study, chemotherapy with DCF induced sustainable progression-free survival in patients with metastatic or unresectable locally advanced recurrent anal squamous cell carcinoma. The protocol-defined primary objective was met, with 47% of patients showing no disease progression in the first 12 months after starting treatment with DCF chemotherapy. The proportion of responding patients in the whole population was high, with 89% of patients achieving a centrally confirmed objective response. Radiological complete responses were observed in 45% of all patients, and pathological complete responses in five (63%) of eight patients who underwent a secondary resection of their metastatic disease. The disease control was as high as 97% according to both central and investigator assessments.

Our results seem to show improved outcomes compared with those previously achieved with CF or carboplatin–paclitaxel combinations, which are currently recommended chemotherapy regimens for patients with advanced recurrent anal squamous cell carcinoma.[4] In a retrospective cohort of 19 patients treated with the CF chemotherapy, only one patient presented a radiological complete response.[7] Although three of these patients were still alive after 4 years, 5 years, and 7 years of follow-up, they were treated in an adjuvant setting (n=1) or underwent complementary surgery or radiotherapy after CF treatment (n=2).[7] Ajani and colleagues[21] reported objective radiological response for CF chemotherapy in three patients with anal squamous cell carcinoma; however, all of them had liver-limited metastases. Since then, several retrospective single institution-based experiences have been published or presented.[8,9,22] CF prescribed in the first-line setting of advanced anal squamous cell carcinoma induced a 34–55% response and median progression-free survival of 5·8–8 months.[8,9] It is noteworthy that complete responses are rare events when using doublet CF chemotherapy. Pooling 92 patients reported in five retrospective studies,[7–9,21,22] complete response was reported in only one of these patients with anal squamous cell carcinoma. Thus, the additional benefit of docetaxel in this population is clear.

Characteristics of the patients included in the Epitopes-HPV02 trial were similar to those in previous retrospective cohort studies[7–9,22] assessing doublet chemotherapies. Only a few patients were HIV positive, and 23% of patients had synchronous metastases compared with 24% in our study population. Moreover, a higher number of metastatic involved sites (67% *vs* 33% with more than one site) and liver metastases (61% *vs* 33%) were reported in our study than in the previous retrospective cohorts.[7–9,22] The high proportion of patients with complete responses achieved with the addition of docetaxel to the CF combination suggests a particular mode of action of taxanes in this setting.[15] Case reports showed efficacy of paclitaxel alone after failure of CF chemotherapy.[15] The combination of carboplatin and paclitaxel has been used outside clinical trials in several centres in the USA, such as the Moffitt Cancer Center in Tampa, FL, and the MD Anderson Cancer Centre in Houston, TX. A retrospective analysis of 12 patients with advanced anal squamous cell carcinoma treated with carboplatin and paclitaxel in the first-line setting showed that 53% patients achieved a response (including three complete responses) and median overall survival of 12·2 months (95% CI 9·36–25·63).[23] The International Multicentre Study in Advanced Anal Cancer Comparing Cisplatin Plus 5 FU versus Carboplatin Plus Weekly Paclitaxel (InterAACT; NCT02051868) is an ongoing prospective randomised phase 2 study of patients with metastatic anal squamous cell carcinoma, comparing the

efficacy of CF chemotherapy with the carboplatin–paclitaxel combination. However, in a retrospective study of patients with advanced anal squamous cell carcinoma, Eng and colleagues[8] reported a lower response (33% *vs* 57%) and shorter median progression-free survival (4 months *vs* 8 months) of the carboplatin–paclitaxel combination compared with the CF regimen, and no complete responses with either regimen.

The clinical interest in integrating paclitaxel into a polychemotherapy regimen was also addressed in a phase 2 trial, in which advanced anal squamous cell carcinoma of various primary sites were treated with a triple combination including paclitaxel, carboplatin, and fluorouracil.[24] Among seven patients with anal squamous cell carcinoma, two partial responses and two complete responses were reported. Long-lasting remission achieved by patients with anal squamous cell carcinoma treated with triplets is in accordance to the results of the Epitopes-HPV02 trial, supporting the development of taxane-based triplet combinations in this disease setting.

A major concern about the DCF regimen is its toxic effects at standard dose. The modified DCF regimen has lower dose intensity than standard DCF for docetaxel and cisplatin (20 mg/m² per week *vs* 25 mg/m² per week) and similar dose intensity for fluorouracil (1200 mg/m² per week *vs* 1250 mg/m² per week). In our trial, modified DCF was recommended for, but not limited to, elderly or frail patients. Patients in the modified DCF population were older, less fit (with a worse ECOG performance status), and more patients had four or more sites involved (27% *vs* 17%) than those in the population treated with standard DCF. The compliance with treatment was better in patients treated with modified DCF, with about 90% of scheduled dose administered for all three drugs compared with about 80% for standard DCF. Although the total cumulative dose of docetaxel and cisplatin was higher in the standard DCF population than in the modified DCF population, no progression-free survival, overall survival, or response advantage was seen for the standard DCF population in our trial. However, the safety profile seemed to favour the modified DCF regimen. Patients treated with modified DCF in our study did not have febrile neutropenia compared with five (14%) patients treated with standard DCF, which is in agreement with previously reported results in other tumour localisations.[14,25,26] In a randomised phase 2 trial[25] of patients with advanced gastric cancer in the first-line setting, modified DCF was associated with fewer adverse events and improved efficacy compared with standard DCF, and thus recommended as a first-line treatment option.The global HRQOL data in the current trial also seemed to favour modified over standard DCF, although not significant. Further data are awaited to compare the health status between standard and modified DCF in patients achieving a long duration of overall survival. Standard DCF has been directly compared with CF chemotherapy in a phase 3 trial[26] in first-line advanced gastric cancer. Grade 3–4 neutropenia was more frequent with DCF than with CF, and 29% of the patients receiving DCF had febrile neutropenia compared with 12% receiving CF. Even if toxic effects were not reported in previous retrospective studies dedicated to patients with advanced anal squamous cell carcinoma, our trial provides important findings about the safety profile of DCF in this disease. The standard DCF regimen induced a higher number of serious adverse events than the modified DCF regimen (69 *vs* 28 events).

14 (21%) of 66 patients received subsequent complementary treatment in our study; this is slightly higher than the 16% (three of 19) reported by Faivre and colleagues[7] and lower than the 43% (33 of 77) reported by Eng and colleagues.[8] Median progression-free survival was not reached in patients who received multidisciplinary treatment. 22 (42%) of 52 patients who did not receive any complementary surgery or radiotherapy were alive and disease-free at 12 months, far exceeding the 11 patients needed to reject the null hypothesis. A median progression-free survival of 10·12 months was achieved in these patients. Moreover, five of eight patients with complementary surgery alone and two of four patients treated with the combination of surgery for their metastases and chemoradiotherapy for their primary tumours had a pathological complete response, supporting the efficacy of DCF regimen.

The majority of our patients were women (82%), which is in accordance to other prospective clinical trials (81% in ACCORD03 and 70% in RTOG-9811).[27,28] A PET scan was done at baseline for all patients. However, we were not able to use PET scan as a confirmatory assessment after DCF because most centres decided to waive the prescription of this PET scan regarding the absence of reimbursement of this examination. The proteins p16 and p53 were proposed as potential prognostic biomarkers in patients with anal squamous cell carcinoma.[15,29] In our trial, however, only a few patients were p16 negative or p53 positive. Age and the number of sites involved were the only prognostic variables in the univariate analysis, but lost significance in the multivariate model.

Our findings showed that DCF chemotherapy allows the expansion of antigen-specific T-cell immune responses. DCF did not decrease absolute lymphocyte count, and HPV and telomerase-specific immune responses were enhanced after DCF chemotherapy. The prognostic value of telomerase CD4 Th1 immunity measured at the end of DCF therapy raises the hypothesis that adaptive immune responses promoted by DCF contribute to the duration of the clinical efficacy observed in this study. The absence of prognostic value of HPV-restricted immune responses raises several hypotheses. Firstly, patients with anal squamous cell carcinoma might be exposed chronically to different strains of HPV viruses in their anal or genital epithelium, leading to a tolerance of HPV-derived antigen and a high level

of specific regulatory T cells, limiting activation of HPV-specific T cells. The landscape of immunogenic tumour antigens recognised by protective memory T cells was previously investigated by Stervanović and colleagues[30] in patients in long-term remission after tumour-infiltrating adoptive T-cell therapy. In this study,[30] immunodominant T-cell reactivities were directed against mutated neoantigens or shared antigens rather than canonical viral-related antigens. Our results raise the hypothesis that hTERT might be such an attractive antigen for anal squamous cell carcinoma.

Although there is no direct comparison between taxane-based doublet and triplet regimens, existing data are in favour of triplet combination regimens. Moreover, prescription of modified DCF could be an attractive option to overcome the major toxicity concerns raised by standard DCF. Non-taxane-based polychemotherapy regimens have previously been evaluated in two clinical trials[31,32] in advanced anal squamous cell carcinoma in which no complete responses were recorded. First, the combination of bleomycin, vincristine, and high-dose methotrexate was given to 15 patients. Unacceptable toxic effects were observed. Three (25%) of 12 patients with measurable disease achieved a partial response.[31] The second trial[32] evaluated the association of mitomycin, doxorubicin, cisplatin, and bleomycin-CCNU in 20 patients with advanced anal squamous cell carcinoma, with 60% achieving a partial response with moderate toxic effects.Another interesting question is the choice between paclitaxel and docetaxel. Although docetaxel has not been evaluated as monotherapy in patients with anal squamous cell carcinoma, it has been shown to induce immunogenic death in cervical cancer cells.[13] In our population, HPV and telomerase-specific immune responses were enhanced after DCF chemotherapy. All these findings suggest that modified DCF is an attractive candidate as a backbone chemotherapy in combination with immunotherapies. Results of InterAACT are awaited to provide more insights in polychemotherapy assessment.

Our study has several limitations. This is a phase 2 trial with a small number of patients, and the DCF regimen was not compared with another chemotherapy regimen. Moreover, no randomisation was done between the two different DCF regimens used in our trial with potential for disbalance in patients' characteristics between the two groups.

Another potential limitation is that only two HIV-positive patients were included in our trial. In view of the increased toxic effects observed in HIV-positive patients with anal squamous cell carcinoma displaying lymphopenia and treated with chemoradiotherapy,[33,34] a CD4 count cutoff above 400 cells per mm³ was selected as an inclusion criterion in Epitopes-HPV02 study. The long-lasting complete response as well as the acceptable safety profile observed in the whole study population and in the two HIV-positive patients with anal squamous cell carcinoma might support the use of modified DCF in HIV-positive patients, although further trials are needed in these patients.

Checkpoint inhibitors including antiprogrammed cell death protein-1 and programmed cell death-ligand 1 (PD1–PD-L1) antibodies (such as nivolumab and pembrolizumab) are promising new treatments for advanced anal squamous cell carcinoma.[35,36] In a prospective phase 2 trial[35] of 37 patients with previously treated metastatic anal squamous cell carcinoma, nine patients (24·0%, 95% CI 15·0–33·0) had responses, including a complete response in two patients.In the phase 1b expansion cohort trial[36] of pembrolizumab for 24 patients with PD-L1 positive advanced anal squamous cell carcinoma, the proportion of patients achieving a response was 17% (95% CI 5·0–37·0), including four partial responses and no complete responses.The estimated 12-month progression-free survival was 20·0% and overall survival was 48·0% for both trials.[35,36] Currently, several trials are evaluating the combination of polychemotherapy and PD1–PD-L1 inhibitors in solid tumours, and future trials of advanced anal squamous cell carcinoma should evaluate the combination of DCF and PD1–PD-L1 inhibitors.

In summary, our results show that the modified DCF regimen could be considered as a new standard of care for patients with metastatic or unresectable locally recurrent anal squamous cell carcinoma with an ECOG performance status of 0–1. Regarding the high risk of serious adverse events of febrile neutropenia despite the primary prophylaxis with G-CSFs, standard DCF cannot be recommended in this situation.

Contributors

SK, TA, MJ, BB, and CB participated in the study design. SK, EF, TA, ES, MJ, FEH, NB-H, SP, M-CK, OB, JD, MZ, FG, AP, CDLF, DS, MD, JT, VV, BB, and CB collected data and recruited patients. SK, MJ, NB, AA, AM, DV, and CB analysed and interpreted the data. LS, OA, and CB did the biomarker analyses. SK, AA, DV, and CB wrote the manuscript. All authors reviewed the report and gave final approval to submit for publication.

Declaration of interests

We declare no competing interests.

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