Atezolizumab plus modified docetaxel, cisplatin, and fluorouracil as first-line treatment for advanced anal cancer (SCARCE C17-02 PRODIGE 60): a randomised, non-comparative, phase 2 study

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Summary

Background The modified docetaxel, cisplatin, and fluorouracil (mDCF) regimen has shown efficacy and safety as first-line treatment for advanced squamous cell carcinoma of the anus, making it a standard regimen. Inhibitors of programmed cell death protein 1 and its ligand, such as pembrolizumab, nivolumab, retifanlimab, avelumab, and atezolizumab, have shown some antitumour activity as monotherapy in advanced squamous cell carcinoma of the anus that is refractory to chemotherapy. This phase 2 study evaluated the combination of mDCF and atezolizumab as first-line treatment in advanced squamous cell carcinoma of the anus.

Methods In this randomised, open-label, non-comparative, phase 2 study, participants from 21 centres (academic, private, and community hospitals and cancer research centres) across France with chemo-naive, metastatic, or unresectable locally advanced recurrent squamous cell carcinoma of the anus, aged 18 years or older, and with an Eastern Cooperative Oncology Group performance status of 0 or 1, were randomly allocated (2:1) to receive either atezolizumab (800 mg intravenously every 2 weeks up to 1 year) plus mDCF (eight cycles of 40 mg per m² docetaxel and 40 mg per m² cisplatin on day 1 and 1200 mg per m² per day of fluorouracil for 2 days, every 2 weeks intravenously; group A) or mDCF alone (group B). Randomisation was done centrally using a minimisation technique and was stratified by age (<65 years *vs* ≥65 years) and disease status. The primary endpoint was investigator-assessed 12-month progression-free survival in the modified intention-to-treat population in group A (35% for the null hypothesis and 50% for the alternative hypothesis). This trial is registered with ClinicalTrials.gov, NCT03519295, and is closed to new participants.

Findings 97 evaluable participants (64 in group A and 33 in group B) were enrolled between July 3, 2018, and Aug 19, 2020. The median follow-up was 26·5 months (95% CI 24·8–28·4). The median age of participants was 64·1 years (IQR 56·2–71·6), and 71 (73%) were female. 12-month progression-free survival was 45% (90% CI 35–55) in group A and 43% (29–58) in group B. In participants with a PD-L1 combined positive score of 5 or greater, 12-month progression-free survival was 70% (95% CI 47–100) in group A and 40% (19–85) in group B (interaction p=0·051) Both groups showed high compliance. Adverse events of grade 3 or higher were observed in 39 (61%) participants in group A and 14 (42%) in group B. The most common grade 3–4 adverse events were neutropenia (nine [14%] participants in group A *vs* five [15%] in group B), anaemia (nine [14%] *vs* one [3%]), fatigue (three [5%] *vs* four [12%]), and diarrhoea (seven [11%] *vs* one [3%]). Serious adverse events occurred in 16 (25%) participants in group A and four (12%) in group B, and these were mDCF-related in seven (11%) participants in group A and four (12%) in group B. Atezolizumab-related serious adverse events occurred in nine (14%) participants in group A, including grade 2 infusion-related reaction in three (5%), grade 3 infection in two (3%), and grade 2 colitis, grade 3 acute kidney injury, grade 3 sarcoidosis, and a grade 4 platelet count decrease each in one participant (2%). There were no treatment-related deaths.

Interpretation Despite a higher incidence of adverse events, combining atezolizumab with mDCF is feasible, with similar dose intensity in both groups, although the primary efficacy endpoint was not met. The predictive value of a PD-L1 combined positive score of 5 or greater now needs to be confirmed in future studies.

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Introduction

Clinical research is increasingly focusing on advanced squamous cell carcinoma of the anus, despite its rarity, due to its rising incidence.[1–3] Approximately 15% of patients present with metastatic disease at diagnosis, and around 25–40% of those with localised disease relapse

Research in context

Evidence before this study

We searched PubMed for clinical trials from database inception to Oct 28, 2023, using the terms "anus neoplasms (Mesh term)" or "anal cancer", "advanced" or "metastatic", "chemotherapy", and "immunotherapy". Our search yielded two study protocol publications evaluating the association of chemotherapy and immunotherapy as first-line treatment of advanced squamous cell carcinoma of the anus . One of these trials is the SCARCE C17-02 PRODIGE 60 trial, which we present here, and the second is the ongoing POD1UM-303/InterAACT-2 phase 3 trial. Additionally, there is another ongoing trial, ECOG-ACRIN 2176, targeting the same population. Previously, the docetaxel, cisplatin, and fluorouracil regimen has shown an unexpectedly high durable complete response rate in the Epitopes-HPV01 trial, which was subsequently validated in the multicentre phase 2 Epitopes-HPV02 trial. Considering the efficacy of anti-PD-1 and anti-PD-L1 immunotherapies in a subgroup of individuals in second or later lines of treatment, we conducted a prospective trial to assess the clinical benefit of combining modified docetaxel, cisplatin, and fluorouracil (mDCF) and atezolizumab in patients with metastatic or unresectable locally recurrent squamous cell carcinoma of the anus in the first-line setting. Two other ongoing phase 3 trials (POD1UM-303 and ECOG-ACRIN 2176) are evaluating the combination of retifanlimab (NCT04472429) and nivolumab (NCT04444921) with carboplatin plus paclitaxel as the chemotherapy backbone.

Added value of this study

This is the first study to evaluate the association of chemotherapy and immunotherapy in patients with advanced squamous cell carcinoma of the anus. This combination was feasible, and study findings confirmed the antitumour activity of the mDCF regimen. However, the added benefit of incorporating immunotherapy might be restricted to individuals with a high expression of PD-L1 combined positive score (≥5%), and these findings warrant confirmation in future trials.

Implications of all the available evidence

Based on the results of this trial, validating PD-L1 expression as a potential predictive biomarker for immunotherapy efficacy in advanced squamous cell carcinoma of the anus is crucial. Exploring novel combinations of immunotherapies such as anti-TIM3 and PD-1 or anti-LAG3 and PD-1 bispecific antibodies with mDCF as well as the combination of hTERT vaccine plus anti-PD-1 and PD-L1 immunotherapy with mDCF could further improve immunotherapy efficacy.

following curative-intent chemoradiotherapy. In patients with distant metastases or a non-resectable local recurrence, the multicentre phase 2 Epitopes-HPV02 trial validated the biweekly modified docetaxel, cisplatin, and fluorouracil (mDCF) regimen as the standard with a 47% complete response rate and 43% 12-month progression-free survival.[6] The biological complete response (complete clearance of human papillomavirus [HPV] circulating tumour DNA [ctDNA]) was met in 61% of participants and was significantly correlated with survival.[7] In addition, mDCF has shown lower toxicity compared with the standard DCF (grade 3–4 toxicity rate of 53% *vs* 83%) at the same efficacy.[6,8] Recently published results of the pooled analysis of Epitopes-HPV01 and Epitopes-HPV02 trials, including 115 evaluable participants with squamous cell carcinoma of the anus treated with DCF, showed 5-year progression-free survival of 25% and overall survival of 44%.[8]

The presence of HPV infection, predominantly genotype HPV-16,[9] is strongly associated with squamous cell carcinoma of the anus. In 90% of the tumours, HPV oncoproteins E6 and E7 are present, either in episomal form or integrated into the DNA. HPV oncoprotein E6 transactivates *hTERT*, leading to the immortalisation by preventing replicative senescence, and also induces the degradation of p53.[10] This loss of normal p53 function confers sensitisation to taxane chemotherapy by increasing G2 and M phase arrest and apoptosis.[11] Additionally, docetaxel has previously been shown to increase endoplasmic reticulum stress and to induce immunogenic death of cancer cells.[12] In ancillary studies of the Epitopes-HPV01 and Epitopes-HPV02 trials,[6,13] DCF exhibited the capacity to induce an antitumour hTERT immune response in addition to effectively reducing the levels of peripheral myeloid suppressive cells.[12] These findings showed a strong correlation with a statistically significant improvement in overall survival, suggesting that mDCF could serve as a potent chemotherapy backbone when combined with immunotherapy in patients with squamous cell carcinoma of the anus.

Immune checkpoint inhibitors targeting programmed death protein 1 (PD-1) and its ligand (PD-L1) have shown some efficacy in a subset of patients with chemorefractory squamous cell carcinoma of the anus.[14–18] The objective response rates observed in published studies ranged from 10% to 24%, and the reported median duration of response exceeded 5 months.[19–22]

Here, we report the results of the SCARCE C17-02 PRODIGE 60 phase 2 trial evaluating the anti-tumour activity and safety of the combination of atezolizumab immunotherapy and mDCF chemotherapy in the first-line setting for patients with advanced squamous cell carcinoma of the anus.[23]

Methods

Study design and participants

This randomised, national, open-label, non-comparative, phase 2 study was conducted in 21 academic, private, and community hospitals and cancer centres across France.

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Participants aged 18 years or older were eligible if they had histologically proven advanced stage squamous cell carcinoma of the anus (stage IV disease with distant metastases or locally advanced recurrence after chemoradiotherapy, non-eligible for salvage surgery due to the extension of the disease), Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, adequate organ functions (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 times upper limit of normal [or ≤5·0 times in the case of known liver metastases], and total bilirubin ≤2·5 times upper limit of normal), and measurable disease according to Response Evaluation Criteria In Solid Tumours version 1.1. Participants who were HIV-positive were eligible if their CD4-positive cell count was ≥400 cells per µL. The main exclusion criteria were previous chemotherapy for metastatic disease, previous immunotherapy at any stage, previous radiotherapy within 28 days before randomisation (14 days if radiotherapy of bone metastases), major surgical procedure within 28 days before initiation of study treatment, inadequate cardiac or respiratory functions, any immunosuppressive therapy (ie, corticosteroids more than 10 mg of hydrocortisone or equivalent dose) within 14 days before the planned start of study therapy, active autoimmune disease, known active CNS metastases, or carcinomatous meningitis. Sex information was recovered from medical records.

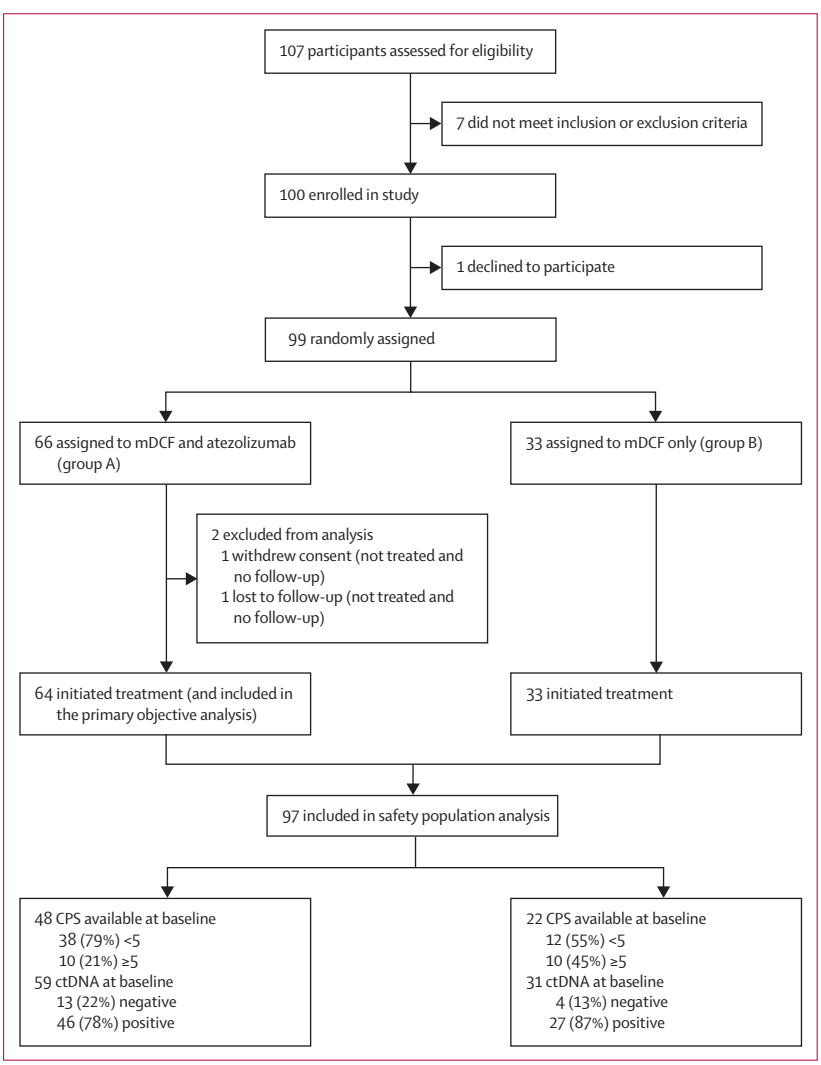


Figure 1: **Trial profile**
CPS=combined positive score. ctDNA=circulating tumour DNA. mDCF=modified docetaxel, cisplatin, and fluorouracil.

The study was developed by the National Institute of Health and Medical Research, Unit 1098, and Clinical Investigational Centre 1431. The trial protocol was approved by the Méditerranée-4 Committee for Protection of Persons on May 15, 2018, and by the French Health Products Safety Agency on April 16, 2018. The study was conducted in accordance with the Declaration of Helsinki and International Conference on Harmonisation Good Clinical Practice guidelines. All participants provided written informed consent.

Randomisation and masking

Participants were randomly assigned (2:1) to receive either first-line mDCF and atezolizumab (experimental group A) or mDCF alone (control group B) by means of an interactive web-based response system using a minimisation technique (hazard compound 0·8), and was stratified by age (<65 years *vs* ≥65 years) and disease status (synchronous metastases *vs* metachronous metastases *vs* locally advanced unresectable disease without metastases). The study was open label, so there was no masking.

Procedures

Participants in both groups received mDCF (docetaxel 40 mg per m² on day 1, cisplatin 40 mg per m² on day 1, and fluorouracil 1200 mg per m² per day for 2 days) by intravenous infusion every 2 weeks for eight cycles. Given the low risk (less than 10%) of febrile neutropenia with the mDCF regimen, support of granulocyte colony-stimulating factor for 4 days (from day 4 to day 7) by subcutaneous injection after mDCF administration was recommended only as secondary prophylaxis. Participants in group A received atezolizumab every 2 weeks for a total of 24 cycles at a fixed dose of 800 mg as a 60 min intravenous infusion preceding mDCF. The first eight cycles were followed by atezolizumab monotherapy for up to 1 year. If treatment was well tolerated at the initial cycle, the infusion duration of atezolizumab could be shortened to 30 min for the subsequent cycles. No dose adjustments were allowed. No premedication treatment was required before infusion of atezolizumab. p16 and HPV status were recorded. HPV ctDNA were assessed at baseline, 2 months, 4 months, and 6 months. HPV

ctDNA levels were determined by digital PCR as previously described (appendix p 2).[7]

Adverse events were measured according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03. Responses to treatment were assessed by Response Evaluation Criteria In Solid Tumours version 1.1. A scheduled blinded central review was done to assess radiographic response. CT was planned at baseline and every 8 weeks until 12 months (or disease progression) from randomisation, and every 12 weeks thereafter. Surgery and palliative radiotherapy of residual metastatic sites were allowed based on the practices of the investigator's centre after week 20. The end-of-treatment visit occurred either 12 months after randomisation or 4 weeks after the last cycle of treatment, if it was stopped prematurely due to progression, toxicity, or a decision made by the physician or patient. In that case, the patients continued to be followed up except for protocol withdrawal. Follow-up visits were performed every 3 months after the end-of-treatment visit until death or at least 3 years after the randomisation date. Laboratory and adverse event monitoring were done at each visit.

Outcomes

The primary outcome was investigator-assessed progression-free survival at 12 months from randomisation in group A. The main secondary outcomes were progression-free survival (time from randomisation to progression or death from any cause, whichever occurred first) according to investigators and central review (participants who did not experience any defined events during the follow-up period and who showed no evidence of progression were censored at the date of last disease evaluation; participants were not censored at the time of complementary curative intent treatments), overall survival (time from randomisation to death of any cause; participants who were alive were censored at the last follow-up visit), objective response rate (participants who had partial or complete radiographic response by Response Evaluation Criteria In Solid Tumours version 1.1), health-related quality of life (HRQoL), and safety.

Other secondary outcomes included: PD-L1 expression (immunohistochemistry) analysis as a potential biomarker (PD-L1 combined positive score [CPS] cutoff levels of 1% and 5% were defined post-hoc); HPV and telomerase-specific T-cell responses before and after treatment, measured by enzyme-linked immunospot assay; peripheral immunological status characterisation,; characterisation of tumour genotyping for HPV, p53, and neoantigens using next-generation sequencing; level of circulating HPV DNA assessed by PCR on cell-free tumour DNA; tumour-infiltrating lymphocytes (isolation or immunohistochemical analysis of Tbet, CD8, Foxp3, and RoR-gt); whole-exome sequencing for determination of mutation-driven neoantigen burden; predictive value of the number and heterogeneity of neoantigens and the role of neoantigen-specific T-cell immunity in comparison with HPV and telomerase immunity; and the impact of sarcopenia on efficacy and tolerability using the L3 skeletal muscle index. Of these other secondary outcomes, only PD-L1 and HPV ctDNA analyses are presented here. Other biomarkers are under investigation and will be reported separately.

See Online for appendix

Statistical analysis

In group A, the objective was to show that 12-month progression-free survival is clearly above a low rate of 35%, which would be considered unsatisfactory. Using a one-arm non-parametric log-rank test survival design with a one-sided type I error of 5% and a statistical power of 80%, it was necessary to randomly assign 62 evaluable participants to group A within a span of 2 years, with at least 1 year of follow-up. The progression-free survival

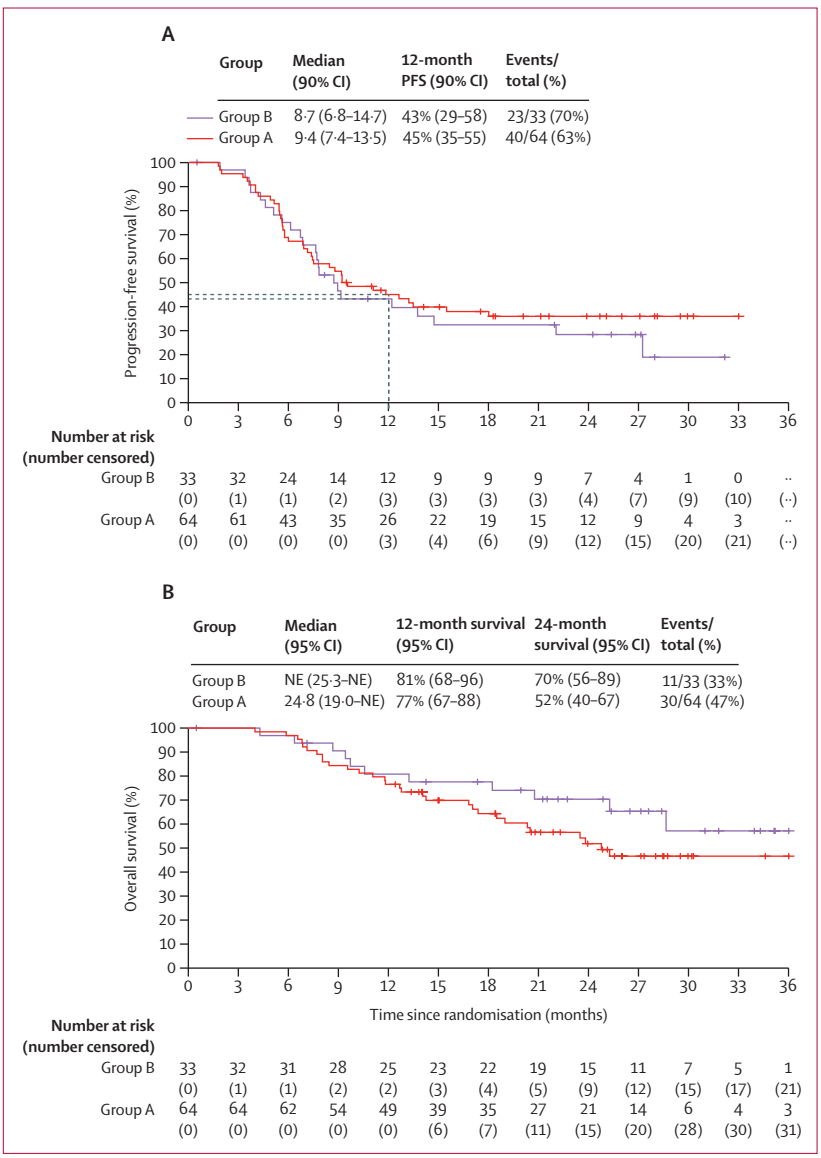
	Group A (n=64)	Group B (n=33)
Median age (IQR), years	63·2 (56·0–71·9)	64·7 (56·4–71·0)
Sex		
Male	18 (28%)	8 (24%)
Female	46 (72%)	25 (76%)
Eastern Cooperative Oncology Group performance status		
0	37 (58%)	24 (73%)
1	27 (42%)	9 (27%)
HIV positive	3 (5%)	1 (3%)
Human papillomavirus positive	59 (92%)	31 (94%)
p16 positive	46 (78%)	27 (87%)
Disease stage		
Synchronous metastasis	26 (41%)	14 (42%)
Metachronous metastasis	25 (39%)	11 (33%)
Locally advanced	13 (20%)	8 (24%)
Previous treatment		
Radio(chemo)therapy	34 (53%)	15 (45%)
Surgery primary tumour	11 (17%)	2 (6%)
Number of metastatic sites		
1	14 (27%)	13 (52%)
2	21 (41%)	6 (24%)
≥3	16 (31%)	6 (24%)
Metastatic sites		
Distant pelvic area	12 (24%)	2 (8%)
Distant lymph node	31 (61%)	11 (44%)
Liver	29 (57%)	12 (48%)
Lung	18 (35%)	8 (32%)
Bone	7 (14%)	2 (8%)
Skin	1 (2%)	1 (4%)
Peritoneum	4 (8%)	5 (20%)
Median follow-up from randomisation (95% CI), months	27·1 (22·2–31·8)	26·0 (22·3–28·5)

Data are n (%) unless otherwise specified. Group A=mDCF plus atezolizumab. Group B=mDCF only. mDCF=modified docetaxel, cisplatin, and fluorouracil.

Table 1: **Baseline demographic and clinical characteristics according to treatment groups**

probabilities at 12 months post-randomisation were 35% for the null hypothesis and 50% for the alternative hypothesis.[6,8] The lowest expected critical value, based on non-parametric exponential estimates to reject the null hypothesis for progression-free survival probability at 12 months, was 47%. Assuming a 5% rate of participant dropout or loss to follow-up, approximately 66 participants had to be randomly assigned to group A. Based on a randomisation ratio of 2:1, 33 participants needed to be randomly assigned to group B, resulting in a total requirement of 99 participants to be randomly allocated.

Group B served to ensure the appropriate calibration for the null hypothesis formulated in group A. No formal statistical comparison was planned between the two groups. The primary analysis was performed on the modified intention-to-treat (mITT) population, ie, including all randomly assigned participants evaluable for the primary outcome, regardless of their eligibility and having received at least one dose of treatment. The safety population included all patients who received at least one dose of treatment in both groups.



(Figure 2 continues on next page)

In group A, the 12-month progression-free survival was estimated using the Kaplan–Meier method and a 90% CI was provided to account for the one-sided type I error of 5% specified in the study design. Both progression-free and overall survival were estimated with the Kaplan–Meier method and described using the median and event-free rates at 12 months with 90% CIs for progression-free survival and 95% CIs for overall survival. The univariate Cox proportional hazards model was used to estimate the hazard ratio (HR) with 95% CI. Follow-up was estimated using the reverse Kaplan–Meier method and was described using the median with its 95% CI.

The time until definitive deterioration (TUDD) for HRQoL using the EORTC-QLQC30 questionnaire was defined as the time from randomisation to the first deterioration by at least 10 points in HRQoL score compared with the baseline, with no further improvement of more than 10 points compared with the baseline score. Survival without HRQoL deterioration (QFS) was defined as the time from randomisation to definitive deterioration of the HRQoL score or death. TUDD and QFS for each HRQoL dimension were analysed using univariate Cox proportional hazards models to estimate the HR with 95% CI and were summarised with forest plots. HRQoL analyses were conducted in the mITT2 population, including the mITT population with baseline HRQoL data available. Exploratory subgroup analyses were summarised with forest plots. Interpretation of the subgroup analysis results was done by evaluating the p value for the interaction term between the studied subgroups and the treatment groups. The interaction was modelled by adding the studied subgroups, the treatment group parameters, and their interaction terms into a single Cox model. A p value of less than 0·1 for the interaction term could be deemed statistically significant and serve as a hypothesis generator for further investigations. The relationship between the baseline value of ctDNA (in its continuous form) and progression-free survival was modelled using the restricted cubic splines method to investigate other relevant cutoffs of interest beyond the positive and negative consideration.

For the primary objective, a one-sided type I error of 5% and a power of 80% were planned. All other p values were two-sided and considered exploratory without any correction for multiple testing. In post-hoc analysis, progression-free survival was estimated by the Kaplan–Maier method according to the presence or absence of complementary treatments.

All statistical analyses were conducted using SAS version 9.4 and R version 4.3.0. The database lock for the

present analysis was performed on Feb 7, 2023. This trial is registered with ClinicalTrials.gov, NCT03519295.

Role of the funding source

The funders had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between July 3, 2018, and Aug 19, 2020, 99 patients were randomly allocated in 21 study sites in France (appendix p 3). Two patients allocated to group A were excluded for analyses: one withdrew consent and the second was lost to follow-up. Neither of these patients received any treatment after randomisation. Among the 97 eligible patients for the activity and safety analyses, 64 were in group A and 33 in group B (figure 1). The median age of patients was 64·1 years (IQR 56·2–71·6), 71 (73%) of 97 patients were female, and 76 patients (78%) had a metastatic disease (table 1). We did not collect data on race or ethnicity according to French recommendations. More patients in group A than in group B had resection of their primary tumour before enrolment (11 [17%] of 64 *vs* two [6%] of 33), and had two or more distant metastatic sites (37 [73%] *vs* 12 [48%]). The median follow-up for all participants was 26·5 months (95% CI 24·8–28·4) and was similar between treatment groups.

The median number of mDCF cycles in both groups was eight (IQR 8–8). The percentages of whole scheduled doses delivered in groups A and B for the first eight cycles were 95·0% versus 97·4% for docetaxel, 90·8% versus 95·2% for cisplatin, and 89·6% versus 96·6% for fluorouracil. The median number of atezolizumab cycles administered in group A was 13·5 (9·5–23·5). The main reason for atezolizumab discontinuation was disease progression in 30 (68%) of the 44 patients who discontinued treatment at any time. Other reasons were serious adverse events in five (11%) patients, investigator's decision in four (9%) patients, non-compliance with protocol in one (2%) patient, and other reasons in four (9%) patients.

12-month progression-free survival was 45% (90% CI 35–55; figure 2A) within the mITT population in group A. As the lower limit of the CI falls below the null hypothesis (35%), this confirms a negative result for the primary objective of the study. The trial did not meet its primary endpoint. For the 33 patients in group B, 12-month progression-free survival was 43% (29–58). Median progression-free survival was 9·4 months (90% CI 7·4–13·5) in group A and 8·7 months (6·8–14·7) in group B. A centralised radiological review confirmed these observations, showing a 12-month progression-free survival of 40% (29–51) in group A versus 43% (27–58) in group B, with median progression-free survival of 10·5 months versus 9·1 months (appendix p 4).

12-month overall survival was 77% (95% CI 67–88) in group A and 81% (68–96) in group B, and 24-month overall survival was 52% (40–67) in group A and 70% (56–89) in group B (figure 2B). Among the 48 participants who received atezolizumab plus mDCF and had PD-L1 CPS available, 12-month progression-free survival was 39% (95% CI 24–62) in the CPS-negative group (n=28), 40% (19–85) for those with CPS 1–4 (n=10), and 70% (47–100) in those with CPS of 5 or greater (n=10; figure 2C). In the exploratory subgroup analysis by CPS score, a differential effect for the study groups was observed when CPS with a threshold of 5 was considered (figure 3, appendix p 13). Notably, the assessment of PD-L1 expression using tumour proportion score did not reveal a specific subset of patients with a distinct sensitivity to atezolizumab (data not shown). Subgroup analyses based on clinical parameters (stage, age, number of metastatic sites, and ECOG performance status at inclusion), did not reveal any differential effect of the treatment groups on progression-free survival (figure 3).

In group A, 47 (75%) of 63 patients had an objective response, with 19 (30%) having a complete response. In group B, 25 (78%) of 32 patients had an objective response, with 16 (50%) having a complete response. The median duration of response was 13·2 months (95% CI 5·2–not evaluable) in group A and 8·0 months (6·1–35·1) in group B (appendix p 5).

Notably, 11 (17%) participants in group A and nine (27%) in group B received complementary treatments such as surgery or radiotherapy for metastasis or primary

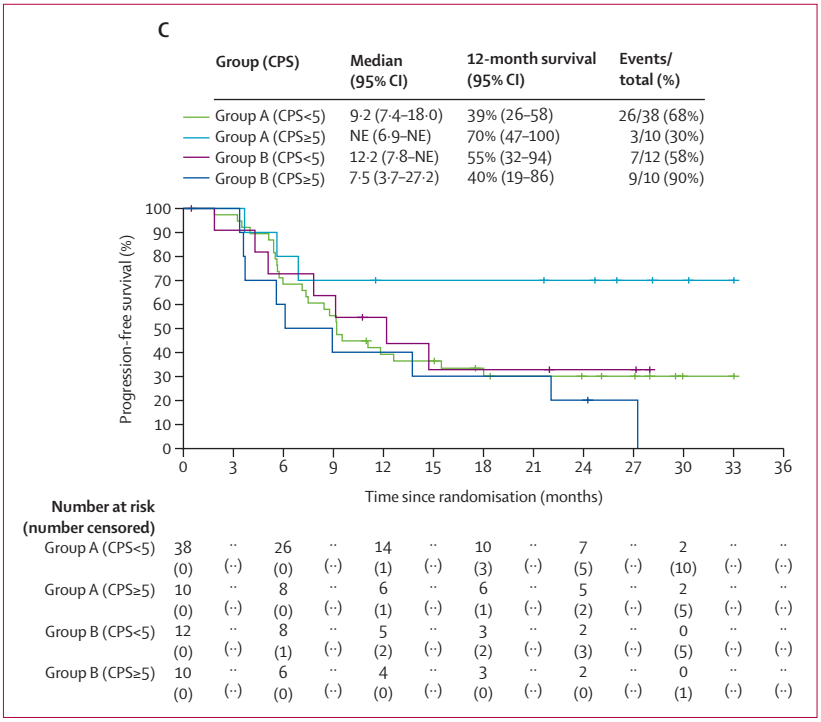


Figure 2: **Kaplan–Meier curves**
Progression-free survival (A) and overall survival (B) according to treatment group, and progression-free survival according to treatment group and CPS using a threshold of 5 (C). Group A=mDCF plus atezolizumab. Group B=mDCF only. CPS=combined positive score. mDCF=modified docetaxel, cisplatin, and fluorouracil. NE=not estimable.

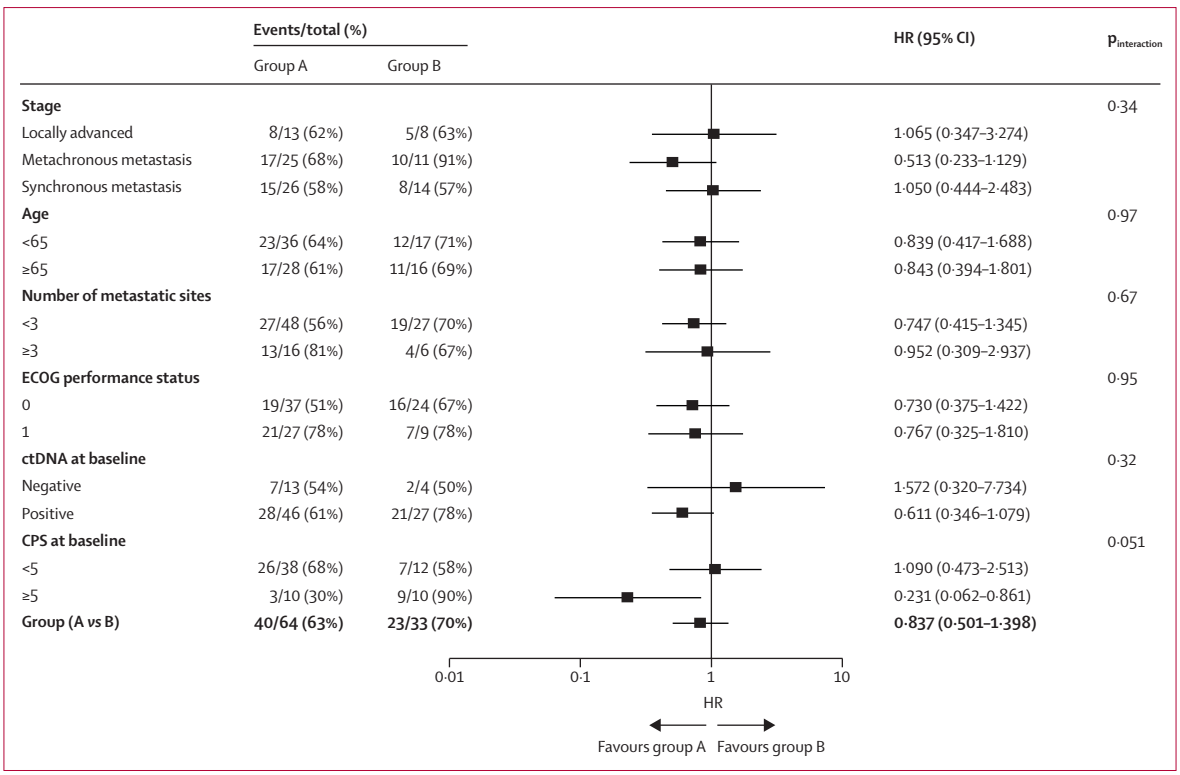


Figure 3: **Subgroup analysis of progression-free survival**
CPS=combined positive score. ctDNA=circulating tumour DNA. ECOG=Eastern Cooperative Oncology Group. Group A=mDCF plus atezolizumab. Group B=mDCF only. HR=hazard ratio. mDCF=modified docetaxel, cisplatin, and fluorouracil.

tumour. The median time from randomisation to the complementary treatment was 5·1 months (IQR 4·3–5·8). Among these individuals, progression of disease occurred in only two (18%) patients in group A, compared with six (67%) in group B (figure 4). Progression-free survival in this subgroup of participants with complementary treatment was 81% (95% CI 60–100) in group A and 39% (16–93) in group B at 2 years, and 81% (60–100) in group A and 0% (0–0) in group B at 3 years. In a post-hoc analysis, no difference in progression-free survival was seen in patients without complementary treatment. However, the difference was significant in patients with complementary treatment (not estimable *vs* 14·7 months, p=0·029; appendix p 6). After progression, 63 patients (40 in group A and 23 in group B) received an anticancer treatment (appendix p 7).

Results for TUDD and QFS for each HRQoL dimension for the 90 patients in the mITT2 population are presented in the appendix (pp 8–9). Similar results between groups were observed, especially for the global quality of life score, and no major differences were identified for the other dimensions.

HPV ctDNA at baseline was measured in 59 (92%) participants in group A and 31 (94%) in group B, and was detected in 46 (78%) and 27 (87%) of these participants. The median copy number of HPV ctDNA per mL of blood was 1216·0 (IQR 53·0–13666·7). 12-month progression-free survival did not differ between patients who had detectable HPV ctDNA levels and those who did not (appendix p 10).

Among the 73 participants who had positive ctDNA at baseline, 69 (95%) had at least one HPV ctDNA follow-up. The complete molecular response rate was 48% in group A (n=28) and 54% in group B (n=15).Improved outcomes in terms of both progression-free survival and overall survival were observed for individuals who had complete molecular response compared with those who did not (appendix p 11).

39 (61%) participants in group A and 14 (42%) in group B had at least one grade 3–4 adverse event. The most common grade 3–4 adverse events were neutropenia in nine (14%) participants in group A versus five (15%) in group B, anaemia in nine (14%) versus one (3%), diarrhoea in seven (11%) versus one (3%), and fatigue in three (5%) versus four (12%). Febrile neutropenia occurred in one (2%) participant in group A and in one (3%) participant in group B (table 2). 16 (25%) participants in group A and four (12%) in group B experienced at least one treatment-related serious adverse event, and these were mDCF-related in seven (11%) participants in group A and four (12%) in group B (table 3). Atezolizumab-related serious adverse events occurred in nine (14%) participants in group A, including grade 2 infusion-related reaction in three (5%), grade 3 infection in two

(3%), and grade 2 colitis, grade 3 acute kidney injury, grade 3 sarcoidosis, and a grade 4 platelet count decrease each in one participant (2%). In group A, five (12%) of 43 participants discontinued treatment following serious adverse event occurrence, compared with one (10%) of ten participants in group B (table 3). There were no treatment-related deaths in either group.

Discussion

The results of this study show that the addition of mDCF to atezolizumab was feasible. The treatment compliance was acceptable and both groups showed good tolerability with similar HRQoL. However, as anticipated, the combination group exhibited higher rates of grade 3–4 toxicities and treatment-related serious adverse events. Nine (14%) participants in the combination group A experienced atezolizumab-related serious adverse events, including four with grade 2 and four with grade 3 adverse events. The sole grade 4 serious adverse event was a decreased platelet count. The incidence of grade 3–4 adverse events with mDCF alone was 36%, which is lower than previously reported rate of 56% in the Epitopes-HPV02 study.[6] Neutropenia was the most frequent grade 3–4 toxicity, observed in nine (14%) participants, even if only two (2%) presented febrile neutropenia.

The study did not meet its primary endpoint. A larger proportion of patients in the combination group had more advanced disease, more previous resection of the primary tumour, and poorer ECOG performance status compared with the group that received chemotherapy alone, mirroring rates observed in our historical data. This underscores the presence of selection bias favouring the control group (appendix p 12). However, albumin, lymphocyte count, and HPV ctDNA burden were well balanced between the study groups. No clear benefit was observed in the different subgroups based on age (≥65 years), number of metastatic sites, ECOG performance status, or stage. However, this study confirms the antitumour activity of triplet mDCF chemotherapy, as previously observed in the Epitopes-HPV01 and Epitopes-HPV02 studies[6,8] and in a 2023 multicentre real-life data study including 247 patients in more than 60 centres.[24]

12-month progression-free survival of 45% in group A and 43% in group B was similar to that in the Epitopes-HPV02 study, in which 47% of participants were alive and free of progression at 12 months. Other efficacy endpoints, such as objective response rate and 12-month overall survival, were also similar to previous data. Therefore, the efficacy of the triplet DCF regimen for achieving durable responses is now supported by three independent prospective studies involving 212 patients: Epitopes-HPV01, Epitopes-HPV02, and the present SCARCE study.[6,8,23]

Anti-PD-1 and PD-L1 immunotherapies have been evaluated in 298 chemorefractory patients with squamous cell carcinoma of the anus across five prospective trials.[14–18] The objective response rate was 40 (13%) of 298 participants in pooled analysis of all five trials, with 12-month progression-free survival of around 15% in all trials. However, in our trial, combining atezolizumab with chemotherapy did not translate into an increased

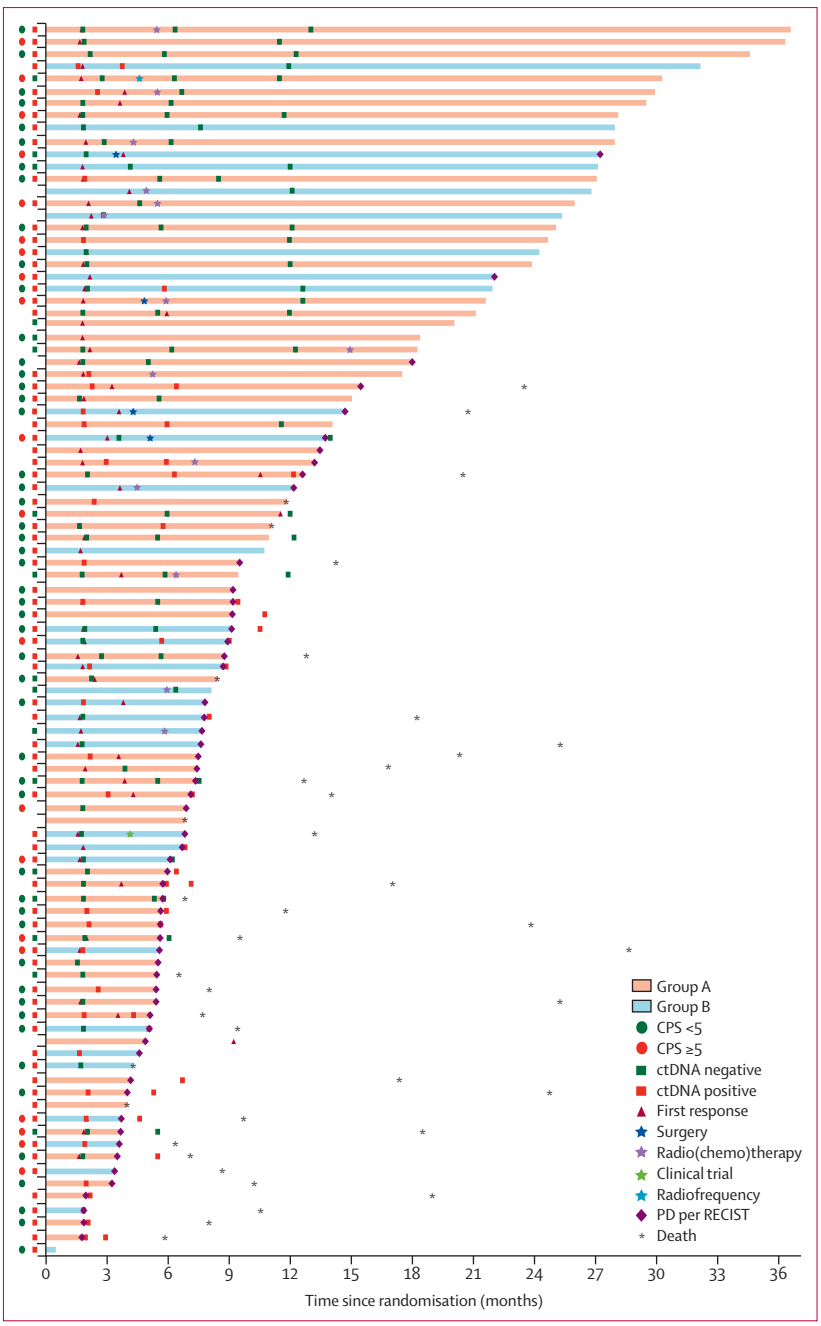


Figure 4: **Swimmer plot for each patient**
Plots are based on participant responses, study and complementary treatments received, baseline CPS score, ctDNA status, and longitudinal ctDNA dynamics follow-up. Clinical trial=inclusion in a clinical trial after the end of the study. CPS=combined positive score. ctDNA=circulating tumour DNA. Group A=mDCF plus atezolizumab. Group B=mDCF only. mDCF=modified docetaxel, cisplatin, and fluorouracil. PD=progressive disease. RECIST=Response Evaluation Criteria in Solid Tumours.

	Group A (n=64)			Group B (n=33)		
	Grade 1–2	Grade 3	Grade 4	Grade 1–2	Grade 3	Grade 4
All adverse events	62 (97%)	35 (55%)	4 (6%)	33 (100%)	12 (36%)	2 (6%)
Fatigue	55 (86%)	3 (5%)	..	27 (82%)	4 (12%)	..
Anaemia	46 (72%)	9 (14%)	1 (2%)	22 (67%)	1 (3%)	..
Diarrhoea	43 (67%)	7 (11%)	..	19 (58%)	1 (3%)	..
Nausea	40 (63%)	2 (3%)	..	18 (55%)	..	..
Neutropenia	27 (42%)	9 (14%)	..	13 (39%)	5 (15%)	..
Peripheral neuropathy	24 (38%)	2 (3%)	..	12 (36%)	..	..
Thrombocytopenia	19 (30%)	2 (3%)	1 (2%)	3 (9%)	..	..
Anorexia	18 (28%)	2 (3%)	..	8 (24%)	1 (3%)	..
Leukopenia	18 (28%)	2 (3%)	..	6 (18%)	2 (6%)	..
Lymphopenia	16 (25%)	3 (5%)	..	8 (24%)	1 (3%)	..
Vomiting	18 (28%)	1 (2%)	..	5 (15%)	..	..
ALP increase	16 (25%)	1 (2%)	..	7 (21%)	..	..
Mucositis	17 (27%)	..	..	5 (15%)	1 (3%)	..
Fever	12 (19%)	..	..	8 (24%)	..	..
ALT/AST increase	15 (23%)	2 (3%)	..	4 (12%)	..	..
Hypokalaemia	12 (19%)	2 (3%)	1 (2%)	3 (9%)	..	..
Creatinine increase	9 (14%)	..	1 (2%)	3 (9%)	..	..
Hyperglycaemia	6 (9%)	..	..	4 (12%)	..	..
Febrile neutropenia	..	1 (2%)	..	..	1 (3%)	..
Allergic reaction	..	1 (2%)	..	..	..	..
Dehydration	..	2 (4%)	..	..	..	..
Hypomagnesemia	..	1 (2%)	..	..	..	..
Other	..	..	..	..	..	2 (6%)

Data are n (%). There were no grade 5 adverse events. ALP=alkaline phosphatase. ALT=alanine transaminase. AST=aspartate transaminase. Group A=mDCF plus atezolizumab. Group B=mDCF only. mDCF=modified docetaxel, cisplatin, and fluorouracil.

Table 2: **Adverse events according to treatment groups**

	Group A (n=64)	Group B (n=33)
mDCF-related serious adverse events	7 (11%)	4 (12%)
Grade 3 acute kidney injury	2 (3%)	..
Grade 3 duodenal haemorrhage	1 (2%)	1 (3%)
Grade 3 febrile neutropenia	1 (2%)	..
Grade 3 hypokalaemia	1 (2%)	..
Grade 3 diarrhoea	1 (2%)	1 (3%)
Grade 4 sepsis	1 (2%)	..
Grade 3 fatigue	..	1 (3%)
Grade 3 infection	..	1 (3%)
Atezolizumab-related serious adverse events	9 (14%)	..
Grade 2 infusion-related reaction	3 (5%)	..
Grade 3 infection	2 (3%)	..
Grade 2 colitis	1 (2%)	..
Grade 3 acute kidney injury	1 (2%)	..
Grade 3 sarcoidosis	1 (2%)	..
Grade 4 platelet count decrease	1 (2%)	..

Data are n (%). Group A=mDCF plus atezolizumab. Group B=mDCF only. mDCF=modified docetaxel, cisplatin, and fluorouracil.

Table 3: **Serious adverse events according to treatment groups**

12-month progression-free survival for the entire population. In the exploratory analysis, the benefit seems to be limited to a subset of participants with high expression of PD-L1. The interaction test was positive even with a modest number of participants, indicating a potential predictivity of CPS of 5 or greater to sensitivity to the addition of atezolizumab. Although this result should be viewed as hypothesis generating, it aligns with findings from the NCI967 and Keynote-158 trials,[14,15] which showed that nivolumab and pembrolizumab monotherapy in second or later lines for patients with squamous cell carcinoma of the anus yielded higher tumour expression of PD-L1 in responders. However, the single-arm design of these studies precludes the interpretation of whether tumour PD-L1 expression might have a predictive value for time-to-event endpoints.

Anti-PD-1 and PD-L1 inhibitors have shown efficacy in advanced squamous cell oesophageal cancer, particularly in individuals with high expression of PD-L1,[22] supporting the use of PD-L1 as a predictive biomarker in squamous cell carcinoma of the anus. In fact, in the CheckMate 648 study, the benefit of adding nivolumab to chemotherapy was more pronounced in patients with PD-L1 of 5% or higher (HR 0·61, 95% CI 0·45–0·83) compared with those with PD-L1 below 5% (0·82, 0·65–1·04).[25] Similar results have been seen in HPV-related tumours such as advanced cervical cancer in the Keynote 826 study, and in head and neck cancer in the Keynote 048 study, where the survival benefit was more pronounced in participants with higher expression of PD-L1 CPS.[26,27] However, only 27% of patients in the current study had CPS of 5 or greater at inclusion, whereas 59% had CPS below 1 and 14% had CPS of 1–4.

Our trial has several limitations that need to be acknowledged. First, this is not a comparative study. A comparative randomised trial in the context of this rare disease was not feasible in an acceptable interval of time. There is a potential risk of imbalance due to the small number of patients in group B. However, to mitigate this risk, participants were stratified with known prognostic factors. Additionally, the obtained results were similar to previous data observed in a cohort of 115 patients treated with mDCF,[8] with no significant differences observed in the subgroup analysis. Second, atezolizumab is an anti-PD-L1 inhibitor, and the negative result observed in this trial might not necessarily apply to anti-PD-1 inhibitors. It is important to consider the presence of tumour PD-L2 expression, as it might confer natural selective resistance to anti-PD-L1 antibodies. PD-L2 expression has already been described in squamous cell head and neck cancer[28] and anal cancer.[29] In fact, avelumab alone and atezolizumab (plus bevacizumab) showed the lowest response rates with immunotherapy in squamous cell carcinoma of the anus in second or later lines, with an objective response rate of only 10% and no complete responses.[17,18] Another possible reason for lack of efficacy of the addition of atezolizumab to mDCF could be selection bias in the randomisation with a relatively small

control group. In this study, the efficacy of the combination was found to be correlated with PD-L1 expression, indicating a synergistic effect in the subgroup of 29% of patients more sensitive to immunotherapy with CPS of 5% or greater. These findings align with data from second or later lines, in which 10–24% of participants are considered responsive to anti-PD-1 and PD-L1 inhibitors in monotherapy.

Another standard chemotherapy regimen comprising carboplatin plus paclitaxel has shown a better tolerance profile with substantially lower severe adverse events compared with cisplatin plus fluorouracil, and is currently under evaluation in combination with nivolumab (NCT04444921) or retifanlimab (NCT04472429) in phase 3 trials.

Another ongoing study (NCT04719988) is evaluating the combination of mDCF and ezabenlimab (an anti-PD-1 antibody) as an induction treatment for locally advanced squamous cell carcinoma of the anus. Promising results observed encourage the development of further neoadjuvant strategies.[30] In addition, vaccination strategies in combination with immunotherapy are now being investigated. In the VolaTIL trial (NCT03946358), hTERT vaccine plus atezolizumab was evaluated in HPV-associated cancers, including second-line or later anal cancer. Preliminary results (unpublished) are encouraging and consistent with other combination trials. In the VolaTIL trial and in the population of CPS 5 or greater in the SCARCE trial, a particular sensitivity to immunotherapy is suggested. Therefore, specific efforts should be made to better understand the eligibility criteria to select patients for immunotherapy in larger, international future phase 3 trials.

In conclusion, the SCARCE C17-02 PRODIGE 60 phase 2 study is the first to evaluate the combination of a chemotherapy regimen and a checkpoint inhibitor in patients with advanced squamous cell carcinoma of the anus. Despite a higher rate of toxicity, the combination of mDCF and atezolizumab proved to be feasible. Although the trial did not meet its primary endpoint, it confirms the antitumour activity and robust safety profile of mDCF, as observed in previous studies, establishing it as a viable first-line treatment for this cancer type. Notably, individuals with tumours of PD-L1 CPS 5 or greater might represent a subgroup that is more sensitive to the atezolizumab and mDCF combination. This, however, requires confirmation in future trials.

Contributors

SK, DV, and CB were responsible for study conceptualisation and design. SK, FG, CdlF, LE, SP, DS, ES, DL-TA, AP, JD, NBH, BB, DT, OB, BC, FEH, LD, M-LG, CB, LM, OD, and MBA were involved in participant inclusion and data collection. SK, FB, MH, AM, DV, and CB analysed and interpreted the data. SK, AM, DV, and CB wrote the original manuscript draft. All authors revised the manuscript critically and had responsibility for the final decision to submit for publication.

Declaration of interests

Genentech provided atezolizumab for the study. SK reports consultancy, advisory roles, and honoraria from Amgen, BeiGene, Boehringer Ingelheim, Merck, MSD, Pfizer, and Servier; and research funding from Boehringer Ingelheim, Bristol Myers Squibb, Novartis, and Roche. FG reports consultancy for Roche, MSD, Merck Serono, Brenus, Engetix, and Odimma; research grants from AstraZeneca, Roche Genentech, Amgen, XBiotech, and Springworks; and travel or accommodation from Amgen, MSD, Roche, and Servier. CdlF reports honoraria from Amgen, AstraZeneca, Bayer, Bristol Myers Squibb, Eisai, Incyte Biosciences, Ipsen, Lilly, Merck Serono, MSD, Pierre Fabre Oncologie, Pfizer, Roche, Sanofi-Aventis, and Servier. ES reports consultancy, advisory roles, and honoraria from Amgen, Astellas, BeiGene, Bristol Myers Squibb, Daichi, Merck, MSD, Pierre Fabre, and Servier; and travel or accommodation from Bristol Myers Squibb, MSD, Pierre Fabre, and Servier. DT reports consulting or advisory board fees from AstraZeneca, Sanofi, Amgen, MSD, BMS, Roche, Servier, and Pierre Fabre; and research funding from Pierre Fabre and Sandoz. OB reports honoraria for speaker or advisory roles from Astellas, Merck Serono, Amgen, Servier, Pierre Fabre, Apmonia Therapeutics, Deciphera, and MSD. DV reports consulting fees from Apmonia Therapeutics, Cellprothera, Novartis, Incyte, Veracyte, and Lysarc. FB reports honoraria for speaker or advisory roles from Astellas, Servier, Incyte, Pierre Fabre, BMS, AstraZeneca, Owkin, and MSD. JT reports honoraria for speaker or advisory roles from Astellas, Roche, Merck Serono, Amgen, Servier, Pierre Fabre, BMS, AstraZeneca, Novartis, Takeda, and MSD. MBA reports honoraria for speaker or advisory roles from Bayer, Incyte, Deciphera, Merck, Servier, BMS, Pierre Fabre, and Amgen. All other authors declare no competing interests.

Data sharing

Deidentified individual-level participant data that support the findings of this study are available from the corresponding author and sponsor on reasonable request. A detailed proposal for how the data will be used is required and we will assess applications on a case-by-case basis. All proposals should be submitted to the corresponding author and sponsor (GERCOR). Data and documents, including the study protocol, clinical study report, and blank or annotated case report forms, will be provided with a secure procedure.

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