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Original Research

Phase 3 trial of sequential versus combination treatment in colorectal cancer: The C-cubed study

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Abstract ***Background:*** An optimal treatment strategy using oxaliplatin and bevacizumab for metastatic colorectal cancer has not been defined. We investigated whether the sequential treatment using fluoropyrimidines with bevacizumab followed by the addition of oxaliplatin at first progression was better than a combination treatment using fluoropyrimidines and

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oxaliplatin with bevacizumab.
Methods: In the sequential treatment, the escalation from fluoropyrimidines plus bevacizumab to fluoropyrimidines plus oxaliplatin with bevacizumab was recommended in case of progressive disease. Time to failure of strategy was the primary end-point, whereas the secondary end-points were overall survival, progression-free survival, overall response rate and safety.
Results: Three hundred patients with previously untreated metastatic colorectal cancer were randomised to receive either the sequential treatment (n = 151) or the combination treatment (n = 149). The sequential treatment was superior to the combination treatment about time to failure of strategy (15.2 months; 95% CI, 12.5–17.2 months vs. 7.8 months: 95% CI, 6.3–9.5 months; $P < 0.001$). However, the median overall survival was 27.5 (95% CI, 24.4 to 32.7) months in the sequential treatment and 27.0 (95% CI, 22.8 to 36.0) months in the combination treatment (hazard ratio, 0.92; 95% CI, 0.66 to 1.28; $P = 0.61$). The overall response rate was 33.1% in the sequential treatment arm and 51.7% in the combination treatment.
Conclusions: The findings support the extension of the sequential treatment starting from fluoropyrimidine plus bevacizumab to selected patients who do not need an objective response to the threatening disease.

1. Introduction

Sequential approaches for untreated unresectable metastatic colorectal cancer (mCRC) treatment using fluoropyrimidines, oxaliplatin, irinotecan and with or without bevacizumab are not associated with significant disadvantages in overall survival (OS) compared with initial combination strategies [1–5]. Oxaliplatin-based regimens are used as the first-line mCRC treatment. Although oxaliplatin is highly effective in combination with fluoropyrimidines, it is often discontinued owing to hypersensitivity or cumulative sensory neuropathy occurring at the threshold dose of $\geq$550 mg/m^2 [6]. Therefore, pre-planned approaches for oxaliplatin usage have been explored in CRC, driven primarily by the desire to obviate or mitigate neurotoxicity [1,2].

Bevacizumab is used in combination therapy for patients with mCRC, irrespective of *RAS* and *BRAF* mutations [7,8]. Bevacizumab can prolong progression-free survival (PFS) [2,7,9], suggesting that it can be used in sequential treatment, with initial disease stabilisation as the primary goal.

In this trial, we tried to confirm whether the timing of oxaliplatin usage affects treatment duration. As oxaliplatin-containing therapies make discontinuation for any reason, including the lack of efficacy and intolerable toxicity, as are often annoying in the prevailing clinical practice. Hence, we set the primary end-point on the superiority of the sequential treatment based on the 'time to failure of strategy (TFS),' which may enable us to examine patients with mCRC who benefit from a given strategy.

2. Materials and methods

2.1. Study design

In this open-label, randomised, multicentre phase 3 trial (C-cubed Study), patients were considered eligible if they (a) had histologically confirmed adenocarcinoma of the colon or rectum with at least one metastatic lesion evaluable by computed tomography (CT) or magnetic resonance imaging, (b) were 20 years or older, (c) had an Eastern Cooperative Oncology Group performance status between 0 and 2, (d) had an adequate haematological function, hepatic function, renal function and coagulation parameters, (e) had an estimated life expectancy >90 days, (f) had no limitation of oral administration and (g) had no previous chemotherapy or radiotherapy for mCRC.

Patients were ineligible if they had (a) history of active double cancer within five years prior to enrolment in the study, (b) history of severe drug hypersensitivity or drug allergy, (c) severe renal failure, haematologic toxicities, diarrhoea, infections, massive pleural effusion and peritoneal fluid, (d) severe or uncontrolled complications, (e) complications owing to cerebrovascular disease or symptoms within one year prior to enrolment in the study, (f) bleeding tendency, coagulopathy, thrombosis, thromboembolism or were receiving anticoagulant drugs, (g) unhealed wound or major surgical procedure within 28 days prior to enrolment in the study, (h) invasive procedure within seven days prior to enrolment in the study, excluding regular blood sampling, drip infusion, endoscopic

examination and central port, (i) aortic aneurysm or aortic dissection, (j) uncontrollable peptic ulcer, (k) concurrent or history of gastrointestinal perforation (within one year prior to enrolment in the study), (l) untreated traumatic bone fracture, (m) uncontrolled hypertension, (n) pre-existing peripheral neuropathy greater than grade 1, or (o) history of adverse events related to dihydropyridine dehydrogenase deficiency. Patients were also ineligible if they (a) had psychological disorders or central nervous system diseases that could hinder study treatments, (b) were pregnant, lactating, with child-bearing potential or refused contraceptive measures, or (c) were judged by the investigator to be unsuitable for any other reason.

All the patients provided written informed consent before trial entry. A contract research organisation (EPS Corporation, Osaka, Japan) was responsible for randomisatioin, data management, monitoring and primary data analysis. Random assignment was organised centrally by fax in a 1:1 fashion using the minimisation method. Köhne index, institution and prior adjuvant chemotherapy (with or without oxaliplatin) served as stratification factors.

This trial is registered at the University Hospital Medical Information Network Centre Clinical Trials Registry (UMIN-CTR), UMIN000015405.

2.2. Procedures and outcomes

Investigators assessed tumour response based on computed tomography or magnetic resonance imaging scans every eight weeks, according to Response Evaluation Criteria in Solid Tumours (RECIST) version 1.1. Progressive disease (PD) was defined as an increase (greater than 20%) in the sum of the longest dimensions of target lesions from baseline (baseline PD) [10]. Exacerbation of the underlying disease and appearance of new lesions (clinical diagnosis of distinct disease progression) were included in the assessment of PD for new non-target lesions.

We adopted TFS as the primary end-point to prove and scrutinise our hypothesis. TFS was conventionally defined as the time to add an agent not in primary regimen, disease progression despite whole therapy, followed by no additional therapy or death [11]. In this trial, to examine the treatment strategy concerning the timing of oxaliplatin usage, the failure of escalation of oxaliplatin by surgery or patient wish in the sequential arm was defined as a censored case because of no function of oxaliplatin.

In this study, there was no crossover administration of capecitabine and 5-fluorouracil. The sequential arm of the trial consisted of two regimens: capecitabine plus bevacizumab or 5-fluorouracil plus bevacizumab. The combination treatment of the trial started with either capecitabine plus oxaliplatin plus bevacizumab or 5-fluorouracil plus oxaliplatin plus bevacizumab regimen, selected by the physician who treated the enrolled patient [12]. Treatment continuation was intended until disease progression or toxicity development. After 12 weeks of treatment, or in case of oxaliplatin-associated toxicity, de-escalation to capecitabine plus bevacizumab or 5-fluorouracil plus bevacizumab was recommended. In case of disease progression after de-escalation, re-escalation was possible only for patients who had received de-escalation of oxaliplatin after 12 weeks of treatment and not due to oxaliplatin-associated toxicity in both arms.

PFS was calculated based on the disease progression or death. In the sequential treatment, fuoropyrimidine plus bevacizumab was escalated after the first progressive disease (PFS1) by adding oxaliplatin; in such patients, the second progressive disease (PFS2) was assessed after treatment intensification. After oxaliplatin induction for 12 weeks, oxaliplatin de-escalation was allowed, while fuoropyrimidine plus bevacizumab was escalated by oxaliplatin re-introduction; in such patients, the third progressive disease (PFS3) was assessed after the second treatment intensified. Thus, in the sequential treatment, three PFSs were estimated independently as PFS1, PFS2, and PFS3 (Fig. 1A).

In the combination treatment, de-escalation from fuoropyrimidine plus oxaliplatin with bevacizumab to fuoropyrimidine plus bevacizumab was possible after 12 weeks of treatment. Thus, PFS1 was the time between randomisation and the first PD with fuoropyrimidine plus oxaliplatin with bevacizumab; PFS2 was determined only in patients who received the scheduled oxaliplatin de-escalation after 12 weeks of treatment. In these patients, PFS2 was the time between the end of PFS1 and PFS2 after re-escalation to fuoropyrimidine plus oxaliplatin with bevacizumab (Fig. 1B).

The secondary objectives were to compare the OS, ORR, PFS1, PFS2 and safety.

2.3. RAS mutation detection

Tumour samples were evaluated clinically for *KRAS* and *NRAS* mutations simultaneously using the MEBGENTM RASKET KIT (Medical Biological Laboratories Co., LTD., Nagoya, Japan) [13]. DNA extraction and analysis were performed as per the manufacturer's protocol. Using the RASKET KIT, we examined twelve types of *RAS* exon 2 (G12S, G12C, G12R, G12D, G12V, G12A, G13S, G13C, G13R, G13D, G13V and G13A), eight types of *RAS* exon 3 (A59T, A59G, Q61K, Q61E, Q61L, Q61P, Q61R and Q61H) and four types of *RAS* exon 4 (K117N, A146T, A146P and A146V) mutations in both *KRAS* and *NRA*S.

2.4. Statistical analysis

Based on published data on fuoropyrimidine plus oxaliplatin with bevacizumab, a median TFS of 11 months

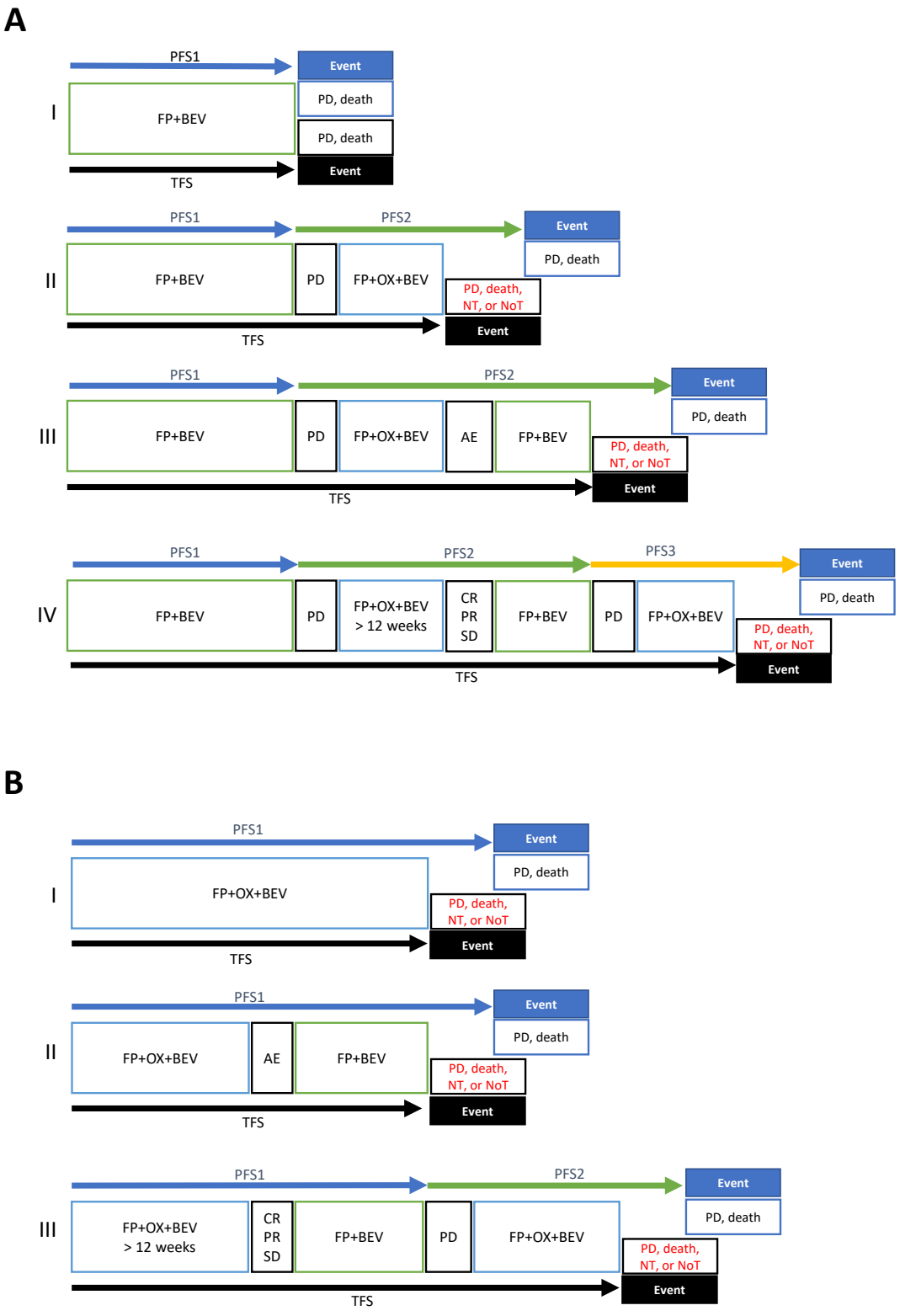

Fig. 1. **Definition of the primary end-point in the sequential treatment (A) and combination treatment (B) arms.** PFS, progression-free survival; FP, fluoropyrimidine; PD, progressive disease; TFS, time to failure of strategy; OX, oxaliplatin; NT, new treatment (not study therapy); NoT, no treatment; AE, adverse event; CR, complete response; PR, partial response; SD, stable disease (all evaluations were according to RECIST 1.1).

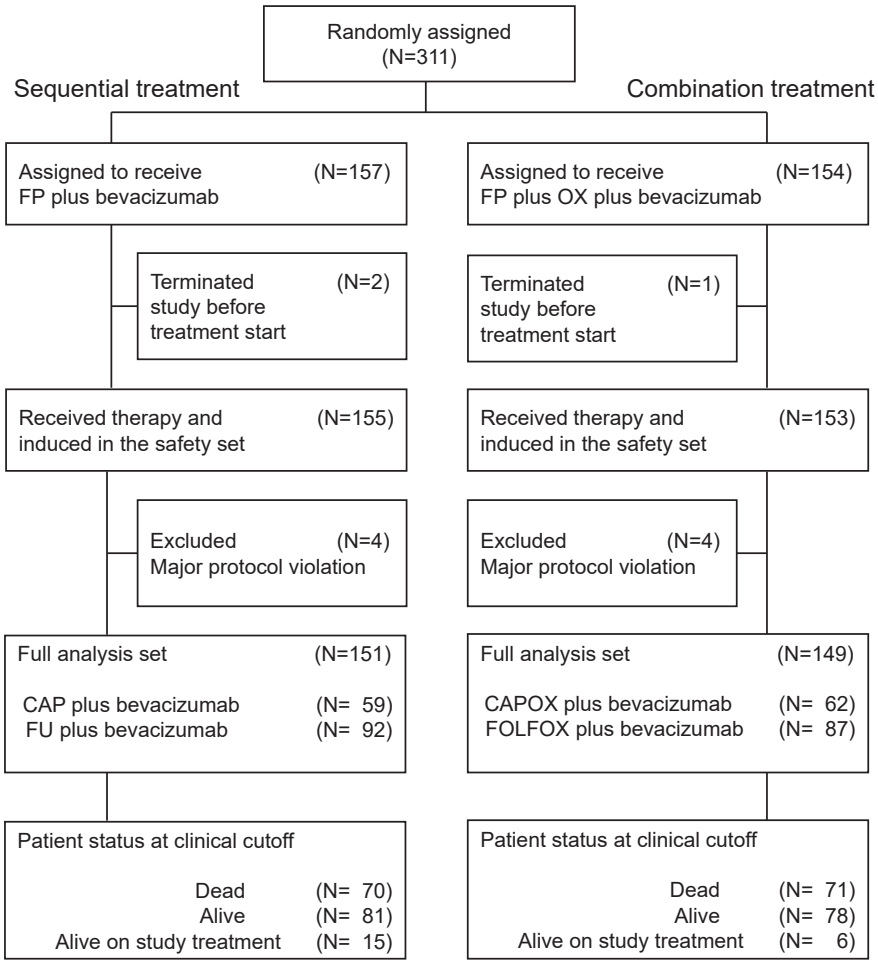

Fig. 2. **Trial profile.** FP, fluoropyrimidines; OX, oxaliplatin; CAP, capecitabine; FU, fluorouracil and leucovorin; CAPOX, capecitabine and oxaliplatin; FOLFOX, fluorouracil, leucovorin and oxaliplatin.

was expected in combination treatment [10,14]. The median PFS (PFS1 of sequential treatment) with fluoropyrimidine plus bevacizumab for the first-line treatment of unresectable CRC was 8.5–10.8 months [15–17]. The median PFS for the second-line fluoropyrimidine plus oxaliplatin plus bevacizumab (PFS2 of sequential treatment) was 5.7 months when bevacizumab was continuously administered during both first- and second-line treatments. Therefore, we estimated a median TFS for sequential treatment as 16 months (10 months for PFS1 plus six months for PFS2). To prove this hypothesis under the assumption of a recruitment period of 2 years and a minimum follow-up period of 1.5 years, 223 events were required to achieve a power of 80% with a two-tailed type 1 error of 0.05. Taking prematurely withdrawn or censored cases into account, the sample size was set at 304 patients.

The primary analysis of TFS was performed with the full analysis set population. The full analysis set included all patients who underwent random assignment, received study treatment and had no significant violation of inclusion or exclusion criteria. The groups were compared using a stratified log-rank test; the stratification factors were used for randomisation. Hazard ratios (HRs) between the groups were estimated using the stratified log-rank test and stratified Cox proportional hazards regression. The Kaplan–Meier method was used to calculate the median and corresponding 95% confidence interval (CI). Response rate and the difference in frequencies, for example, event in TFS, symptomatic toxicities, and patients treated with oxaliplatin, between the study arms, were compared using Fisher's exact test. The mean total dose of oxaliplatin between the arms was calculated using Wilcoxon signed-rank test. The prespecified subgroup analysis included sex, age group, surgery on the primary tumour, site of the primary tumour, stage at diagnosis, sites of metastases, number of metastases, Köhne index, Eastern Cooperative Oncology Group performance status, adjuvant chemotherapies, *RAS* mutational status, regimen and total cumulative dose of oxaliplatin, as variables. Analyses and graphics were performed and

generated using SAS (version 9.4; SAS Institute, Cary, NC). All *P*-values were estimated without adjusting the significance level using a two-sided test.

3. Results

3.1. Study population

We randomly assigned 311 patients who visited 81 centres across Japan 1st between December 2014, and 16th September 2016; 11 patients were found ineligible and excluded from the analysis (Fig. 2). Of the remaining 300 patients, 151 were assigned to the sequential treatment arm and 149 to the combination treatment arm (Table 1). 21 patients were still on treatment (15 on sequential treatment and six on combination treatment).

3.2. Efficacy

The primary analysis of TFS was based on 224 (74.7%) of 300 events. The median TFS in the sequential treatment arm was 15.2 (95% CI, 12.5 to 17.2) months and that in the combination treatment arm was 7.8 (95% CI, 6.3 to 9.5) months (HR, 0.49; 95% CI, 0.37 to 0.64; $P < 0.0001$; Fig. 3A). At the secondary end-points, the median OS was 27.5 (95% CI, 24.4 to 32.7) months in the sequential treatment and 27.0 (95% CI, 22.8 to 36.0) months in the combination treatment (HR, 0.92; 95% CI, 0.66 to 1.28; $P = 0.61$; Fig. 3B). Also, the PFS1 was not different between the groups; the median PFS1 was 12.0 (95% CI, 9.3 to 13.3) months in the sequential treatment and 12.9 (95% CI, 11.2 to 18.5) months in the combination treatment (HR, 1.31; 95% CI, 0.93 to 1.84; $P = 0.12$; Fig. 3C). Regarding PFS2, 68 of the 151 (45.0%) patients in the sequential treatment qualified for PFS2 calculation, and the median PFS2 was 5.8 months (95% CI, 4.4 to 6.5). Only 6 (4.0%) of the 149 patients in the combination treatment qualified for PFS2 calculation, and their median PFS2 was 3.0 months (95% CI, 0.7 months to not evaluated; Fig. 3D). The PFS3 of only four patients in the sequential treatment was calculated (median PFS3 was 3.5 [95% CI, 1.9 to 3.5]) months.

The number of patients with PD or death was not different between the arms, but the number of patients who required the addition of an agent not included in the primary regimen or who were followed by no additional therapy was higher in the combination treatment (n = 76, 51.0%) than in the sequential treatment (n = 51, 33.8%, Table 2). The addition of an agent not included in the primary regimen or follow-up with no additional therapy was mainly caused by adverse events (Supplementary Table 1).

All patients enrolled in the combination treatment received oxaliplatin. In the sequential treatment, successful oxaliplatin escalation according to the protocol was observed only in 74 (49.0%) of the 151 patients. Regarding TFS calculation, among the 74 patients with successful oxaliplatin escalation, 66 patients (89.2%) were counted as the event, and only eight (10.8%) were censored due to failure to oxaliplatin escalation or reinduction according to protocol. In contrast, among the remaining 77 patients (51.0%) with failure to oxaliplatin escalation, 46 patients (59.7%) were censored mainly due to failure to oxaliplatin escalation or reinduction according to protocol, failure to reinduction after conversion surgery and ongoing protocol treatment at the time of data cutoff (March 31, 2018).

Table 1
Baseline characteristics of randomised and eligible patients.

	Sequential treatment (n = 151)	Combination treatment (n = 149)
Age		
Median age at randomisation, years (range)	68 (41–87)	70 (43–89)
> 70 years, n (%)	72 (47.7)	77 (51.7)
Sex, n (%)		
Male	83 (55.0)	91 (61.1)
Female	68 (45.0)	58 (38.9)
Performance status, n (%)		
0	129 (85.4)	119 (79.9)
1	21 (13.9)	26 (17.5)
2	1 (0.7)	4 (2.7)
Adjuvant chemotherapy, n (%)		
Yes (with oxaliplatin)	9 (6.0)	9 (6.0)
Yes (without oxaliplatin)	10 (6.6)	9 (6.0)
No	132 (87.4)	131 (88.0)
Köhne index, n (%)		
High	33 (21.9)	33 (22.2)
Intermediate	40 (26.5)	37 (24.8)
Low	78 (51.7)	79 (53.0)
Site of primary tumour, n (%)		
Right colon	45 (29.8)	50 (33.6)
Left colon	45 (29.8)	51 (34.2)
Rectum	54 (35.8)	41 (27.5)
Unknown	7 (4.6)	7 (4.7)
Surgery on primary tumour, n (%)		
Yes	121 (80.1)	124 (83.2)
No	30 (19.9)	25 (16.8)
Stage of disease, n (%)		
Synchronous	105 (69.5)	110 (73.8)
Metachronous	46 (30.5)	39 (26.2)
Metastatic site (s), n (%)		
Liver	88 (58.3)	85 (57.0)
Liver only	31 (20.5)	32 (21.5)
Lung	60 (39.7)	46 (30.9)
Peritoneum	34 (22.5)	35 (23.5)
Lymph nodes	46 (30.5)	50 (33.6)
Number of metastatic organs, n (%)		
1	76 (50.3)	80 (53.7)
>2	75 (49.7)	69 (46.3)
Fluoropyrimidine, n (%)		
Capecitabine (CAP)	59 (39.1)	62 (41.6)
5-fluorouracil (FU)	92 (60.9)	87 (58.4)
RAS mutation status, n (%)		
Wild	59 (39.1)	58 (38.9)
Mutant	83 (55.0)	85 (57.1)
Unknown	9 (6.0)	6 (4.0)

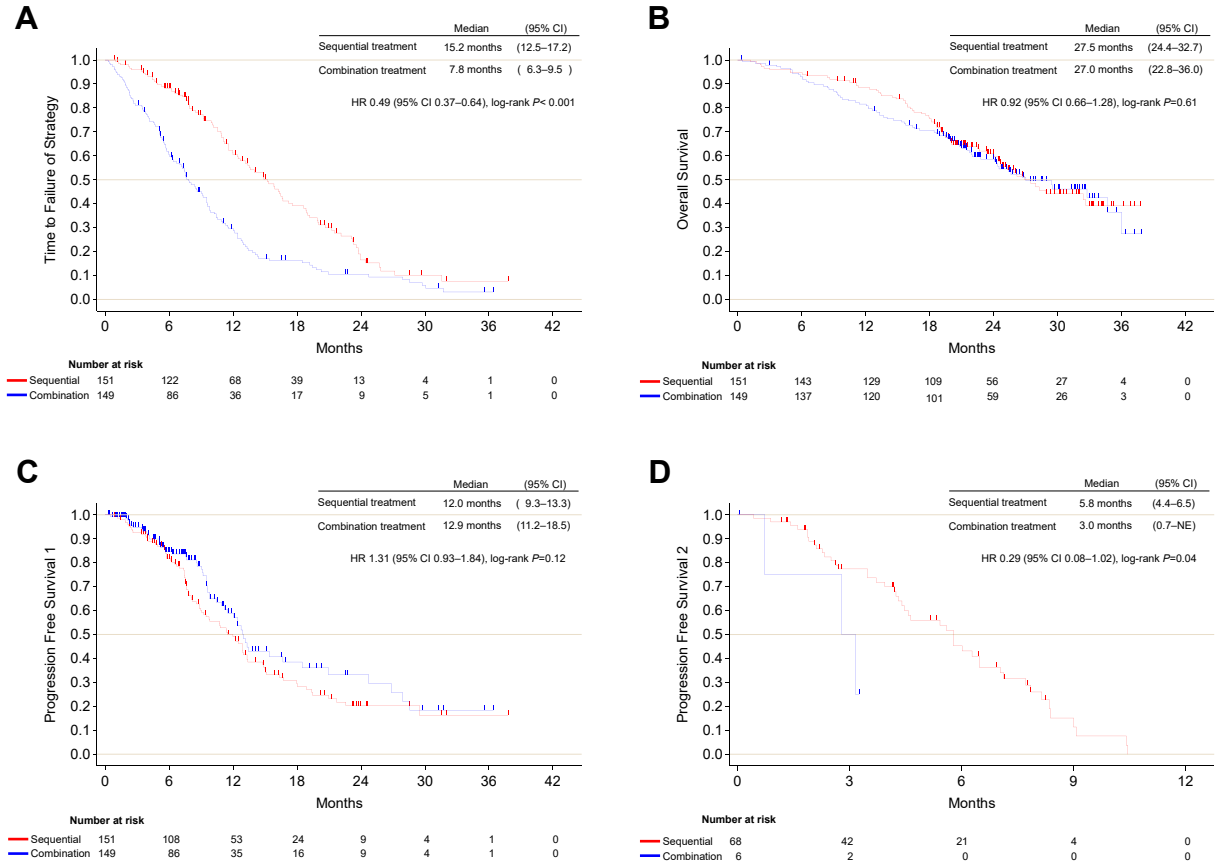

Fig. 3. **Kaplan–Meier estimates of the full analysis set.** Kaplan–Meier estimates TFS (A), OS (B), PFS1 (C), and PFS2 (D), according to the treatment groups. HR, hazard ratio; N.E., not evaluated; OS, overall survival; PFS, progression-free survival; TFS, time to failure of strategy.

However, of the patients with failure to oxaliplatin escalation, 11 patients received oxaliplatin at subsequent treatment (Supplementary Table 1). Overall, 85 (56.3%) of 151 patients in the sequential arm received oxaliplatin treatment at the time of data cutoff.

In the combination treatment, while all 149 participants received oxaliplatin initially, the number of patients who succeeded in scheduled de-escalation followed by re-escalation along with the protocol was only six (4.0%). Of note, there was no difference between both study arms concerning the proportion of patients who could receive subsequent therapies (106 patients [70.2%] in the sequential treatment and 107 patients [71.8%] in the combination treatment) and the variety of treatment regimens. The details of subsequent treatment regimens are summarised in Supplementary Table 2.

The mean total cumulative dose in patients in whom oxaliplatin was successfully introduced in the sequential treatment was 590 mg/m^2 oxaliplatin. All patients were initially treated with oxaliplatin in the combination treatment arm, and the mean total cumulative dose was 767 mg/m^2 (Table 2).

Although the median TFS in the combination treatment was shorter than that in the sequential treatment, more patients in the former presented an ORR than in the latter (77 [51.7%] versus 50 [33.1%] patients; $P = 0.002$). However, there was no difference in the disease control rate between the groups (128 [85.9%] patients in the combination treatment versus 137 [90.7%] patients in the sequential treatment; $P = 0.21$, Table 2).

3.3. Safety

Overall toxicity was not different between the arms. The incidence of grade 3/4 neutropenia was more in the combination treatment than in the sequential treatment ($P = 0.02$), whereas all-grade thrombocytopenia was more frequent in the sequential treatment ($P = 0.04$; Table 3). Both all-grade and grade 3/4 sensory neuropathy was more frequent in the combination treatment than in the sequential treatment ($P < 0.001$ and $P = 0.002$, respectively). The all-grade hand-foot syndrome and nose bleeding were higher in the sequential treatment than in the combination treatment ($P < 0.001$

Table 2
The number of events and censored in time to failure of strategy, number and total dose of patients treated with oxaliplatin and efficacy.

	Sequential treatment (n = 151)	Combination treatment (n = 149)	*P*- value
Time to failure of strategy (TFS), n (%)			
Event	97 (64.2)	127 (85.2)	<.0001
Censored	54 (35.8)	22 (14.8)	
Reasons for event of TFS, n (%)			
Progressive disease	43	47	
Death	3	4	
Addition of an agent not in primary regimen or followed by no additional therapy	51	76	
Reason for censored of TFS, n (%)			
Failure to oxaliplatin escalation or reinduction according to protocol	30	8	
Failure to reinduction after Conversion surgery	12	9	
Complete response (CR)	2	4	
Ongoing protocol treatment	10	0	
Others	0	1	
Subsequent treatment, n (%)			
Yes	106 (70.2)	107 (71.8)	0.2631
No	40 (26.5)	41 (27.5)	
Unknown	5 (3.3)	1 (0.7)	
Number of patients treated with oxaliplatin, n (%)			
	74 (49.0)	149 (100)	<0.0001
Total cumulative dose of oxaliplatin (mg/m2)			
Mean (95% CI)	590 (513–667)	767 (700–834)	0.003
Efficacy, n (%)			
Complete response (CR)	3 (2.0)	8 (5.4)	
Partial response (PR)	47 (31.1)	69 (46.3)	
Stable disease (SD)	87 (57.6)	51 (34.2)	
Progressive disease (PD)	12 (7.9)	13 (8.7)	
Not evaluated	2 (1.3)	8 (5.4)	
Overall response rate (CR + PR)	50 (33.1)	77 (51.7)	0.002
95% CI (%)	25.7–41.2	43.4–59.9	
Disease control rate (CR + PR + SD)	137 (90.7)	128 (85.9)	0.21
95% CI (%)	84.9–94.8	79.3–91.1	

and $P = 0.02$, respectively). The incidence of grade 3/4 proteinuria was higher in the sequential treatment than in the combination treatment ($P = 0.02$).

3.4. Subgroup analysis

Exploratory subgroup analyses were performed for TFS, OS and PFS1 Supplementary Fig. 1 to 3). Although an exploratory analysis of TFS showed a better tendency in sequential treatment, the OS analysis showed that both treatments have comparable effects. Based on the PFS1, the combination treatment showed a better outcome in patients treated with fluoropyrimidines, patients with synchronous metastases and patients with metastases not limited to the liver than in patients who received sequential treatment.

Regarding the predictive effect of the *RAS* status, treatment type had no significant effect on the TFS. In the sequential treatment arm, the median TFS for *RAS* wild-type was 16.2 months (95% CI, 11.2 to 20.0) and that for the *RAS* mutant was 15.2 months (95% CI 12.4 to 16.7; HR = 0.74 [95% CI, 0.48–1.13], $P = 0.16$; Fig. 4A). In the combination treatment arm, the median TFS of *RAS* wild-type was 7.8 months (95% CI, 6.4 to 9.5) and that of the *RAS* mutant was 8.0 months (95% CI 5.5 to 9.8; HR = 0.85 [95% CI, 0.59 to 1.23], $P = 0.39$; Fig. 4B). In contrast, no statistical significance was observed in the sequential treatment arm (median OS of *RAS* wild-type was not reached [95% CI, 24.4 months to not evaluated] and median OS of the *RAS* mutant was 26.9 months [95% CI, 23.4 to 32.7], HR = 0.70 [95% CI, 0.42 to 1.18], $P = 0.18$; Fig. 4C). In the combination treatment arm, a better prognosis was observed in patients with *RAS* wild-type (median OS: 34.7 months, 95% CI, 25.4 months to not evaluated), than in those with the *RAS* mutant (median OS: 24.3 months, 95% CI, 19.5 to 29.5) (HR = 0.54 [95% CI, 0.32 to 0.91], $P = 0.02$; Fig. 4D).

4. Discussion

This trial demonstrated the superiority of the sequential treatment in terms of the primary end-point TFS with the HR of 0.49 compared with that of the combination

Table 3
Adverse events.

	Sequential treatment (n = 151)	Combination treatment (n = 148)	*P*-value
Overall toxicity, n (%)			
All grades	151 (100)	148 (100)	1
Grade 3–4	67 (44.4)	59 (39.9)	0.48
Non-haematological adverse events, n (%)			
Mucositis			
All grades	68 (45.0)	59 (39.9)	0.41
Grade 3–4	2 (1.3)	2 (1.4)	1
Anorexia			
All grades	79 (52.3)	81 (54.7)	0.73
Grade 3–4	3 (2.0)	5 (3.4)	0.5
Nausea			
All grades	49 (32.5)	57 (38.5)	0.28
Grade 3–4	1 (0.7)	3 (2.0)	0.37
Vomiting			
All grades	15 (9.9)	15 (10.1)	1
Grade 3–4	1 (0.7)	3 (2.0)	0.37
Diarrhea			
All grades	48 (31.8)	38 (25.7)	0.25
Grade 3–4	2 (1.3)	3 (2.0)	
Alopecia			
All grades	22 (14.6)	13 (8.8)	0.68
Grade 3	0 (0.0)	0 (0.0)	1
Fatigue			
All grades	67 (44.4)	76 (51.4)	0.25
Grade 3–4	1 (0.7)	6 (4.1)	0.06
Haematological adverse events, n (%)			
Neutropenia			
All grades	51 (33.8)	64 (43.2)	0.1
Grade 3–4	2 (1.3)	10 (6.8)	0.02
Febrile neutropenia			
All grades	0 (0)	1 (0.7)	0.5
Grade 3–4	0 (0)	0 (0)	1
Thrombocytopenia			
All grades	47 (31.1)	63 (42.6)	0.04
Grade 3–4	1 (0.7)	0 (0)	1
Special interest events, n (%)			
Sensory neuropathy			
All grades	79 (52.3)	118 (79.7)	<0.0001
Grade 3–4	0 (0.0)	9 (6.1)	0.002
Hand-foot syndrome			
All grades	108 (71.5)	69 (46.6)	<0.0001
Grade 3–4	2 (1.3)	1 (0.7)	1
Allergic reaction			
All grades	7 (4.6)	6 (4.1)	1
Grade 3–4	1 (0.7)	0 (0.0)	1
Hypertension			
All grades	81 (53.6)	85 (57.4)	0.56
Grade 3–4	30 (19.9)	19 (12.8)	0.12
Proteinuria			
All grades	80 (53.0)	66 (44.6)	0.17
Grade 3–4	18 (11.9)	6 (4.1)	0.02
Gastrointestinal haemorrhage			
All grades	5 (3.3)	1 (0.7)	0.21
Grade 3–4	1 (0.7)	1 (0.7)	1
Nose bleeding			
All grades	39 (25.8)	22 (14.9)	0.02
Grade 3–4	2 (1.3)	0 (0)	0.5
Thromboembolic events			
All grades	2 (1.3)	5 (3.4)	0.28
Grade 3–4	0 (0.0)	2 (1.4)	0.24

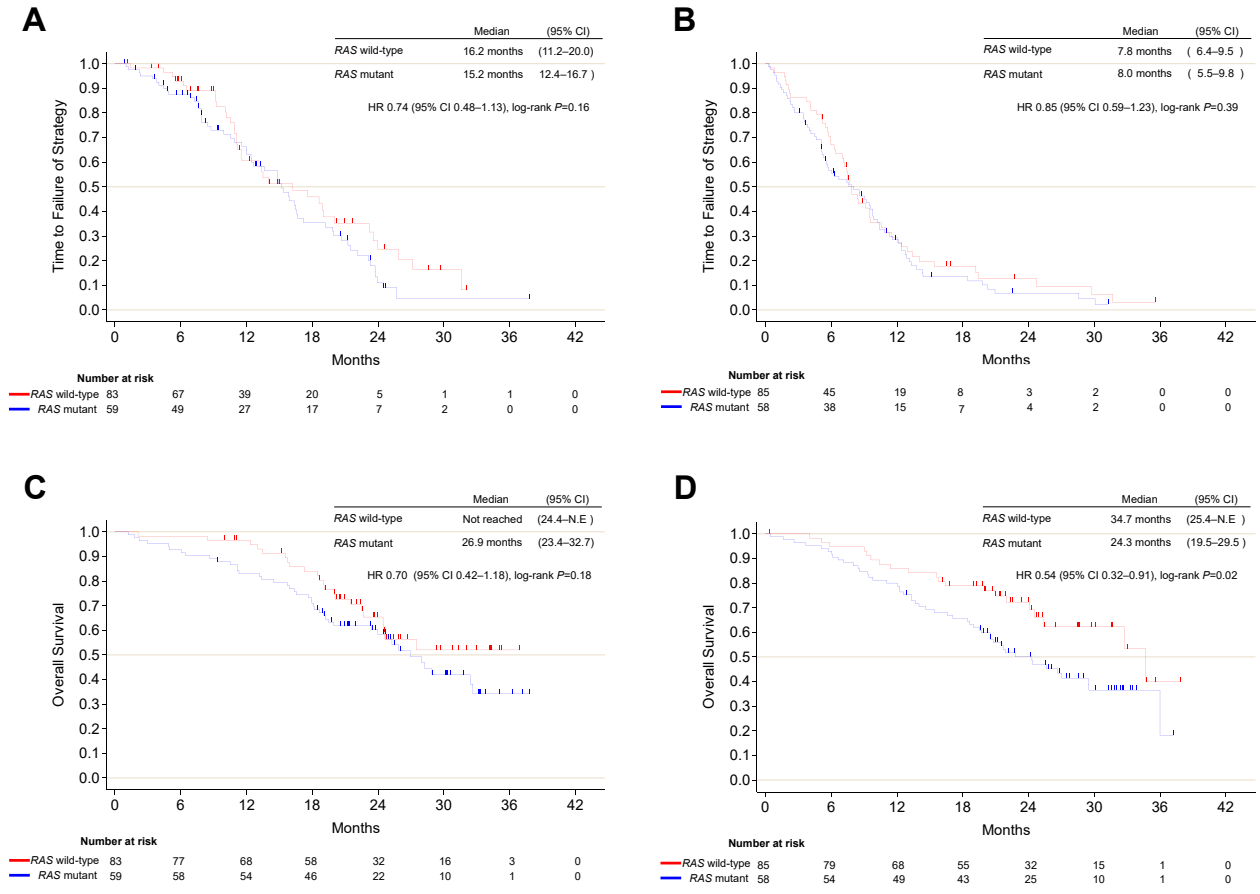

Fig. 4. **Kaplan–Meier estimates of the *RAS* mutational status.** Kaplan–Meier estimates TFS in the sequential treatment (A) and combination treatment (B) arms. Kaplan–Meier estimates OS in the sequential treatment (C) and combination treatment (D) arms. HR, hazard ratio; N.E., not evaluated; OS, overall survival; TFS, time to failure of strategy.

treatment. Additionally, as expected by previous studies, there was no significant difference in the OS or PFS1 between the groups.

The following observations characterise this study. First, we adopted 'TFS' as the primary end-point. As the aim of this trial was to confirm whether the timing of oxaliplatin usage affects treatment duration, events of TFS were counted by not only PD or death but also at the time of an addition of an agent not included in the study regimen or termination of first-line chemotherapy, which were mainly owing to adverse events. TFS would be shorter than PFS1 if the participants experienced intolerable adverse events or did not enter the escalation or scheduled oxaliplatin de-escalation strategy. Indeed, in the combination treatment, events on TFS caused by adverse events were frequently observed (56 patients in the combination treatment versus 31 patients in the sequential treatment).

The association between TFS and PFS1 in this trial could be easy to follow by referring to Fig. 1. There are four possible treatment strategies (Fig. 1A, I to IV) in the sequential treatment arm depending on how oxaliplatin is used. In all four treatment strategies, TFS is estimated constantly longer than PFS1 (TFS > PFS1). In contrast, although the combination arm possesses three possible treatment strategies, two out of three treatment strategies (Fig. 1B, I and II) could estimate TFS shorter than PFS1 (TFS < PFS1). In the combination treatment, the scheduled oxaliplatin de-escalation strategy (Fig. 1B, III) is the only strategy to make the following inequality (TFS > PFS). However, this planned strategy was not acceptable to most participants, and only six patients succeeded in this scheduled strategy according to the protocol. Therefore, TFS in the combination arm might be destined to be closer to the time over 9–12 cycles of the treatment, which is the duration to acquire cumulative oxaliplatin toxicities which require discontinuation while the tumour is still oxaliplatin sensitive [20].

Second, the participants were relatively older than other clinical trials [3,18,19]. However, the population of elder participants was similar to the XELAVIRI trial

that compared sequential versus combination approaches using fluoropyrimidines, bevacizumab and irinotecan [5].

Third, only 49.0% of participants experienced oxaliplatin induction in the sequential arm. Interestingly, while the trials performed in the pre-antibody era showed over 50% of participants received sequential escalation treatment [1–3], the XELAVIRI trial in the antibody era performed in the minority (37.7%) of patients received an additional agent, irinotecan, in the sequential arm. The XELAVIRI trial reported that, finally, 63.2% of patients in the sequential arm received irinotecan at some time during therapy [5]. Our study found that considering subsequent treatment, 56.3% of patients in the sequential treatment received oxaliplatin, which is similar to the pre-antibody and antibody era trials [1–3,5].

During the scheduled treatment, the mean total cumulative dose in patients in whom oxaliplatin was successfully introduced in the sequential treatment was 590 mg/m^2. Instead, all participants were initially treated with oxaliplatin in the combination treatment, and its mean total cumulative dose was 767 mg/m^2. As the mean total cumulative dose of oxaliplatin ranged from 765 to 922 mg/m^2 in recent trials that used initial oxaliplatin-based combination treatment with or without antibodies in the first-line for mCRC [18,19], the total cumulative oxaliplatin dose in combination treatment would be acceptable.

The advantage of combination treatment was that its ORR was higher than that of sequential treatment (51.7% in combination treatment versus 33.1% in sequential treatment). A similar tendency was observed in the XELAVIRI trial (54% in combination treatment versus 37% in sequential treatment) [5] and phase 3 trials in the pre-antibody era (41–58% in combination treatment versus 20–28% in sequential treatment) [1–3]. Our results regarding the ORR suggested that patients who require tumour shrinkage should be treated with upfront combination therapies. However, similar to the FFCD 2000-05 trial [3], our study demonstrated that combination treatment resulted in higher objective responses, which did not translate into higher resection rates (conversion surgery was performed in nine [6.0%] of combination and 12 [8.0%] of sequential treatment).

Whereas the overall rates of grade 3/4 adverse events were similar in both arms, the rates of some individual adverse events differed between the regimens. While the combination treatment was associated with a higher incidence of grade 3/4 neutropenia than sequential treatment, the frequency of all-grade neutropenia was not significantly increased in the combination treatment arm. The rates of all-grade and grade 3/4 neurosensory toxicities were significantly higher in the combination treatment arm than in the sequential treatment arm. The incidence of bevacizumab- and fluoropyrimidines-induced toxicities, proteinuria, nose bleeding and the hand-foot syndrome was higher in the sequential treatment arm than in the combination treatment arm. The difference could explain those in exposure times to bevacizumab and fluoropyrimidines. Indeed, the treatment duration was twice as long as TFS in the sequential treatment arm than in the combination treatment arm.

The molecular subgroup analysis of the CAIRO3 study compared capecitabine plus bevacizumab maintenance treatment with observation treatment after six cycles of capecitabine, oxaliplatin and bevacizumab demonstrated no significant differences in treatment effect among tumours categorised according to *RAS*/*BRAF* mutational status [21]. However, such molecular subgroups represented a prognostic effect; for example, a good prognosis was demonstrated in the order of *RAS*/*BRAF* wild-type, *RAS* mutant and *BRAF* mutant tumours. Our trial demonstrated that the *RAS* wild-type had a better OS, suggesting that the *RAS* mutational status in tumours holds prognostic value irrespective of the treatment strategy.

This study has some limitations. First, the scheduled oxaliplatin de-escalation strategy was unacceptable in most participants, making TFS shorter in the combination arm. Second, this study lacked information on the *BRAF* mutation, which leads to a worse prognosis in the cytotoxic-based chemotherapies [5, 21, 22]. However, as this study is a randomised control study and the population of patients with *BRAF* mutation is around 6% in stage IV CRC in Japan [23, 24], the lack of *BRAF* mutational status would not influence the study results.

Considering with trials performed in the pre-antibody and antibody era, including this trial, the sequential treatment starting from fluoropyrimidines with or without bevacizumab would eventually result in about 60% of patients being able to add additional agents, irinotecan or oxaliplatin. This study demonstrated no significant difference in the OS or PFS1 between the groups, but the rate of neurosensory toxicities annoying daily life was higher in the combination treatment. Thus, the extension of the sequential treatment to selected patients, where an objective response is not needed to control the threatening disease.

Data availability statement

The data supporting this study's findings are available on request to JSWOG (http://www.jswog.org/mail.html).

Ethics approval

This study was approved by the central ethics committee of a third-party organisation, NPO MINS Research Ethics Committee (reference number; 140,206), following the principles of the Declaration of Helsinki.

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Disclosure

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Author's contributions

RI, TN, HO, SN, HT, YM, YaS, KI, YoS and HM collected the data and recruited patients. TN and MS analysed and interpreted the data. TN and RI wrote the manuscript. YaS assisted with the interpretation of all data. TN, HM, MO, and YY designed the study. All authors revised and approved the manuscript.

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2022.04.009.

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