Effect of neutropenia on survival outcomes of patients with metastatic colorectal cancer receiving trifluridine/tipiracil plus bevacizumab

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Abstract. Trifluridine (FTD)/tipiracil (TPI) plus bevacizumab (Bev) is a promising late-line treatment in metastatic colorectal cancer (mCRC). Although chemotherapy-induced neutropenia (CIN) is a well-known predictor of FTD/TPI efficacy, whether CIN is a predictive marker of efficacy for FTD/TPI + Bev remains unclear. Thus, the present study aimed to investigate the clinical outcomes of FTD/TPI + Bev and the predictive markers of its efficacy. Clinical data of patients with mCRC who received FTD/TPI + Bev at the Cancer Institute Hospital between January 2017 and August 2020 were retrospectively collected. Disease control rate (DCR), progression-free survival (PFS), overall survival (OS) and safety were assessed. In addition, subgroup analyses of prognostic and predictive efficacy markers were performed. In total, 94 patients (median age, 60.0 years; age range, 32-82 years; 37 men and 57 women) were included in the present study. The DCR was 44.7%, the median PFS time was 2.9 months (2.3-4.1 months) and the median OS time was 10.0 months (7.3-11.1 months). Grade 3 or 4 CIN within the first cycle of treatment occurred in 27.7% of patients, which was significantly associated with a longer PFS time than those who did not develop CIN [3.8 months (2.3-8.4 months) vs. 2.7 months (1.8-4.0 months); P=0.008]. Furthermore, the DCR was higher in patients with grade 3 or 4 CIN within the first cycle of treatment than those without CIN (61.5 vs. 38.2%; P=0.07). Multivariate Cox regression analysis revealed that grade 3 or 4 CIN within the first cycle of treatment are independent predictors for a longer PFS time (P=0.01). Taken together, the results of the present study suggest that grade 3 or 4 CIN within the first cycle of treatment are early predictors of the efficacy of FTD/TPI + Bev.

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Abbreviations: BSC, best supportive care; CIN, chemotherapy-induced neutropenia; CR, complete response; DCR, disease control rate; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; PR, partial response; SD, stable disease

Key words: trifluridine tipiracil, bevacizumab, colorectal cancer, chemotherapy-induced neutropenia, predictive factor

Introduction

Trifluridine (FTD)/tipiracil (TPI) are nucleoside antineoplastic agents that are used in 1:0.5 molar ratio as a novel oral treatment (1). FTD is an active anticancer agent that possibly mediates its effect by inducing DNA dysfunction through direct uptake into DNA after oral administration. TPI specifically inhibits thymidine phosphorylase, the enzyme that degrades FTD, increasing the bioavailability of FTD (1-3). FTD/TPI has a different mechanism of action from conventional antineoplastic agents such as 5-fluorouracil. Moreover, a preclinical study has shown the effect of FTD/TPI on tumors with low susceptibility to pyrimidine fluoride-based antineoplastic agents (4). Following a phase III trial comparing FTD/TPI monotherapy and best supportive care (BSC) (5,6), FTD/TPI was approved for patients with metastatic colorectal cancer (mCRC) with refractory to conventional standard chemotherapy in Japan, the US, and the Europe Union (7-9). One of the predictive factors of FTD/TPI efficacy is chemotherapy-induced neutropenia (CIN), which is well known as the most common adverse event of this drug (10). The predictive nature of CIN is attributed to a dose-response relationship between FTD exposure and neutropenia, in agreement with the findings of a dose-escalation study that a higher rate of neutropenia at higher FTD and TPI doses implies greater drug efficacy (10). In addition, FTD/TPI plus bevacizumab (Bev) is an alternative treatment option as a third- or later-line chemotherapy for patients with mCRC, as this treatment is safe and leads to a higher disease control rate (DCR) and longer progression-free survival (PFS) and overall survival (OS), than FTD/TPI monotherapy. Nevertheless, there are only a

few phase I/II (11-13) or randomized phase II trials (14) investigating the efficacy of FTD/TPI + Bev. Moreover, because the PFS following this salvage-line treatment is still low, predictors of early treatment efficacy are important to help optimize treatment strategies; however, the efficacy predictors of FTD/TPI + Bev remain unclear. Therefore, this study aimed to evaluate the clinical outcomes of FTD/TPI + Bev and to explore predictors of its efficacy.

Materials and methods

Patients. This is a retrospective cohort study in a single institute in Japan. Patients with mCRC who received FTD/TPI + Bev at the Cancer Institute Hospital, Japanese Foundation for Cancer Research, from January 2017 to August 2020 were enrolled. This study was approved by the Institutional Review Board of the Japanese Foundation for Cancer Research (Tokyo, Japan, registry number 2020-1017). The protocol was described on the hospital website, and subjects were provided the opportunity to opt-out; therefore, no additional consent was required from patients. All the data were readily available and not taken specifically for this study. All methods were performed in accordance with the Declaration of Helsinki.

Treatment schedule. FTD/TPI (35 mg/m^2) was administered orally twice daily, after breakfast and dinner, for 5 days a week for 2 weeks, followed by a 14-day rest, and then Bev (5 mg/kg) was administered via intravenous infusion for 30 min every 2 weeks. This treatment cycle was repeated every 4 weeks until tumor progression or unacceptable toxicity occurred or at the patient's request. Dose reductions and treatment discontinuations were performed owing to toxicity, disease progression, or based on the physician's decisions (11).

Study endpoints. Tumor response was assessed by computed tomography using the RECIST guidelines, v1.1. Complete response (CR) was defined as the complete disappearance of all detectable evidence of disease as determined using total body computed tomography. Partial response (PR) was defined as a minimum of 30% decrease in the sum of target lesion diameters. Stable disease (SD) was defined as everything between a 30% decrease and a 20% growth in tumor size. Progressive disease was defined as a minimum of 20% increase in the sum of target lesion diameters. Objective response rate (ORR) implied the proportion of patients who showed CR or PR to therapy, and DCR indicated the proportion of patients who showed CR, PR, or SD response to therapy. PFS was defined as the time from the first day of treatment to either the first objective evidence of disease progression or death from any cause. OS was defined as the time from the first day of treatment until the time of death. Toxicity was graded according to the Common Toxicity Criteria for Adverse Events v4.0, both within the first cycle and at all periods of treatment. Neutrophils were measured during the first cycle treatment or just before the initiation of second cycle treatment, which was defined as CIN within the first cycle of the treatment. We also evaluated the relationship between clinical outcomes of FTD/TPI + Bev and neutropenia within the first cycle.

Statistical analysis. PFS and OS were estimated using the Kaplan-Meier method, and the statistical significance of the correlation between the clinical outcome and clinical parameters was assessed using the log-rank test. We compared the categorical characteristics by conducting the Pearson's χ^2 tests. Statistical tests provided two-sided P values, with $P<0.05$ considered significant. In the Cox proportional hazard analysis, factors with $P<0.05$ in the univariate analysis were included in the multivariate analysis (backward stepwise methods). Statistical analyses were conducted using the EZR statistical software (Saitama Medical Center, Jichi Medical University, Saitama, Japan) 1.41 based on R and R commander (15).

Results

Patient characteristics. The characteristics of 94 patients with mCRC who received FTD/TPI + Bev are summarized in Table I. The median age at the time of data collection was 60 (range, 32-82) years. Of the 94 patients, 37 were male (39.3%). The lung was the most frequent site of metastasis (73.4%), followed by the liver (59.6%), lymph node (48.9%), and peritoneum (42.6%) at the start of FTD/TPI + Bev. Fifty-seven patients (60.5%) harbored *RAS* mutants in their tumor tissues. There were no significant differences in clinical characteristics between mCRC patients with and without grade 3 or 4 neutropenia within the first cycle of treatment (Table SI).

Toxicity. We reviewed the adverse events (AEs) of patients with mCRC who received FTD/TPI + Bev. Grade 3 or 4 AEs in all treatment periods occurred in 56 patients (59.6%). AE occurrences in 94 patients with mCRC are summarized in Table II. There were no treatment-related deaths. The most common grade 3 or 4 AEs were neutropenia (51.1%), anemia (13.8%), and thrombocytopenia (6.4%), respectively. Febrile neutropenia occurred in one patient (1.1%). Grade 3 or 4 neutropenia in the first cycle of treatment occurred in 26 patients (27.7%). There were significant differences in the incidence of leucopenia, anemia, thrombocytopenia, and hypertension between patients with and without grade 3 or 4 CIN within the first cycle of treatment (Table SII).

Survival endpoints and factors associated with survival. The median PFS was 2.9 months (2.3-4.1), and the median OS was 10.0 months (7.3-11.1) (Fig. 1). The ORR and DCR were 0% and 44.7%, respectively (Table III). Patients with grade 3 or 4 CIN within the first cycle of treatment had significantly longer PFS (3.8 months [2.3-8.4] vs. 2.7 months [1.8-4.0], P=0.008) (Fig. 2A) and tended to have longer OS (11.1 months [8.3-15.5] vs. 9.0 months [6.9-11.1], P=0.19 (Fig. 2B). Furthermore, there were no complete nor partial response. The DCR in patients with grade 3 or 4 CIN within the first cycle of treatment was higher than in patients without grade 3 or 4 CIN (61.5 and 38.2%, P=0.07; Table III).

Univariate and multivariate analyses of predictors of clinical outcomes. In the univariate Cox proportional hazard analysis, liver metastasis, lymph node metastasis, and grade 3 or 4 CIN within the first cycle of treatment were

Table I. Patient demographics and clinical characteristics (n=94).

Characteristic	Number of patients, n (%)
Median age at enrollment, years (range)	60 (32-82)
Sex	
Male	37 (39.3)
Female	57 (60.7)
ECOG PS	
0	64 (68.1)
1	24 (25.5)
2	6 (6.4)
Primary site	
Right-sided colon	26 (27.7)
Left-sided colon	68 (72.3)
Metastatic site	
Lung	69 (73.4)
Liver	56 (59.6)
Lymph node	46 (48.9)
Peritoneal	40 (42.6)
Other	26 (27.7)
RAS status in tissue	
Wild-type	37 (39.4)
Mutant	57 (60.6)
Time from the start of first-line chemotherapy, months	
<18	32 (34.0)
≥18	60 (63.8)
Unknown	2 (2.2)
Number of prior regimens	
1	0 (0.0)
2	68 (72.3)
3	22 (23.4)
4	4 (4.3)
Prior regimens	
Fluoropyrimidine	94 (100.0)
Irinotecan	94 (100.0)
Oxaliplatin	93 (98.9)
Angiogenesis inhibitor	94 (100.0)
Anti-EGFR antibodies	18 (19.1)
Number of metastasis	
1	10 (10.6)
>1	84 (89.4)

ECOG PS, Eastern Cooperative Oncology Group Performance Status; Other, brain, bone, ovary and adrenal glands; *RAS*, rat sarcoma viral oncogene homolog; EGFR, epidermal growth factor receptor.

Table II. Incidence of adverse events during treatment period.

Adverse event	Any grade, n (%)	Grade 3 or 4, n (%)
Anemia	45 (47.9)	13 (13.8)
Neutropenia	71 (75.5)	48 (51.1)
Thrombocytopenia	18 (19.1)	6 (6.4)
Anorexia	20 (21.3)	0 (0.0)
Vomiting	15 (16.0)	0 (0.0)
Nausea	47 (50.0)	0 (0.0)
Diarrhea	16 (17.0)	0 (0.0)
Febrile neutropenia	1 (1.1)	1 (1.1)
Hypertension	12 (12.8)	0 (0.0)
Proteinuria	33 (35.1)	0 (0.0)

predictive factors for PFS. PS and liver metastasis were predictive factors for OS (Tables IV and V). Moreover, liver and lymph node metastases were independent predictive factors for a shorter PFS, while CIN was an independent predictive factor for a longer PFS (liver metastasis: HR 1.82, 95% CI 1.17-2.83, P=0.007; lymph node metastasis: HR 2.23, 95% CI 1.40-3.54, P=0.0007; grade 3 or 4 CIN within the first cycle of treatment: HR 0.51, 95% CI 0.3-0.86, P=0.01) in the multivariate analysis. Furthermore, liver metastasis and performance status were independent predictive factors for a shorter OS (liver metastasis: HR 2.31, 95% CI 1.34-3.98, P=0.002; PS: HR 2.26, 95% CI 1.29-3.97, P=0.004) in the multivariate analysis (Tables IV and V).

Previous reports of clinical outcomes. A summary of previous prospective and retrospective reports of FTD/TPI + Bev for patients with mCRC receiving salvage-line therapy is presented in Table VI (11,14,16-20). In these reports, the median PFS time was 3.7-6.8 months and the median OS time was 8.6-14.4 months. In addition, grade 3 or 4 neutropenia occurred in 38.9-72.0% of all cases. The clinical outcomes of the present study were comparable to previous reports (11,14,16-20). A summary of previous prospective and retrospective reports of FTD/TPI monotherapy for patients with mCRC is presented in Table SIII (5,6,21-28). In these reports, the median PFS time was 2.0-2.5 months and the median OS time was 5.3-9.0 months. Furthermore, grade 3 or 4 neutropenia occurred in 14.3-50.0% of all cases.

Discussion

In the present study, we explored the clinical outcomes of FTD/TPI + Bev and the predictive factors of its efficacy. To our knowledge, this is the first study to demonstrate that CIN within the first cycle of treatment is an indicator for the efficacy of FTD/TPI + Bev in multivariate analysis of a large number of cases. FTD/TPI + Bev showed enhanced activity against colorectal cancer xenografts compared with FTD/TPI alone (29). Moreover, clinical data from the phase I/II C-TASK FORCE study and the phase II study conducted by Pfeiffer *et al* (11,14) showed that treatment with FTD/TPI + Bev induced promising antitumor activity with manageable toxicity in advanced mCRC refractory or intolerant to standard therapies. A summary of previous prospective and retrospective reports of FTD/TPI + Bev

Table III. Summary of antitumor response.

Variable	Total number of patients (n=94)	Patients with grade 3 or 4 neutropenia within the first cycle of treatment (n=26)	Patients without grade 3 or 4 neutropenia within the first cycle of treatment (n=68)	P-value
Best overall response, n (%)				
Complete response	0 (0.0)	0 (0.0)	0 (0.0)	-
Partial response	0 (0.0)	0 (0.0)	0 (0.0)	-
Stable disease	42 (44.7)	16 (61.5)	26 (38.2)	-
Progressive disease	45 (47.9)	10 (38.5)	35 (51.5)	-
Not evaluated	7 (7.4)	0 (0.0)	7 (10.3)	-
Disease control rate, %	42 (44.7)	16 (61.5)	26 (38.2)	0.07

-, not available.

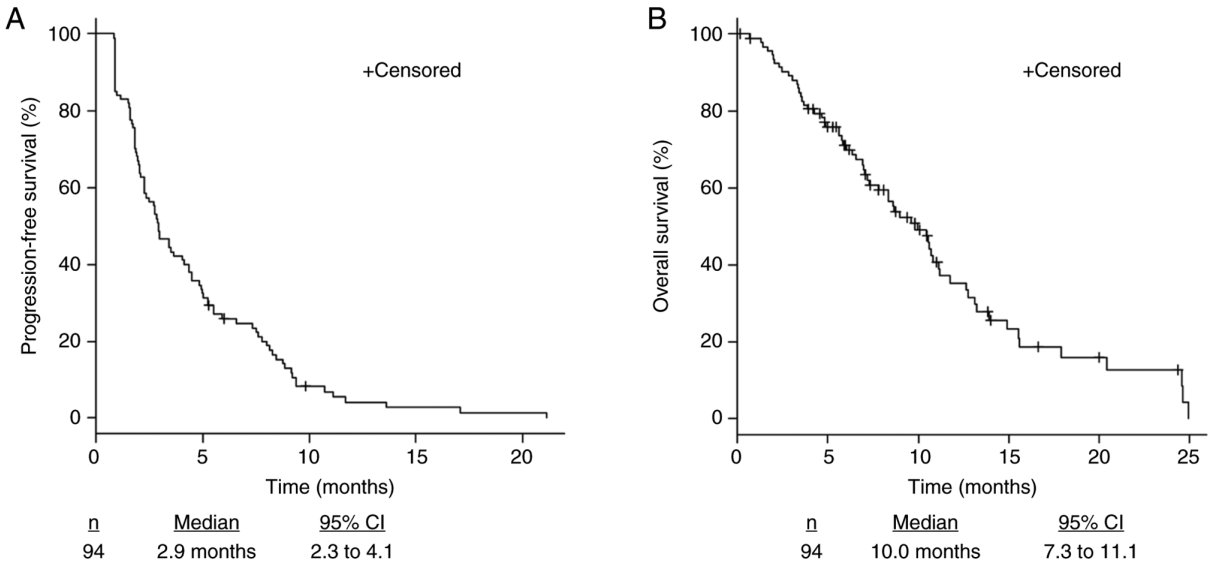

Figure 1. Kaplan-Meier estimates of (A) progression-free survival and (B) overall survival in all patients. CI, confidence interval.

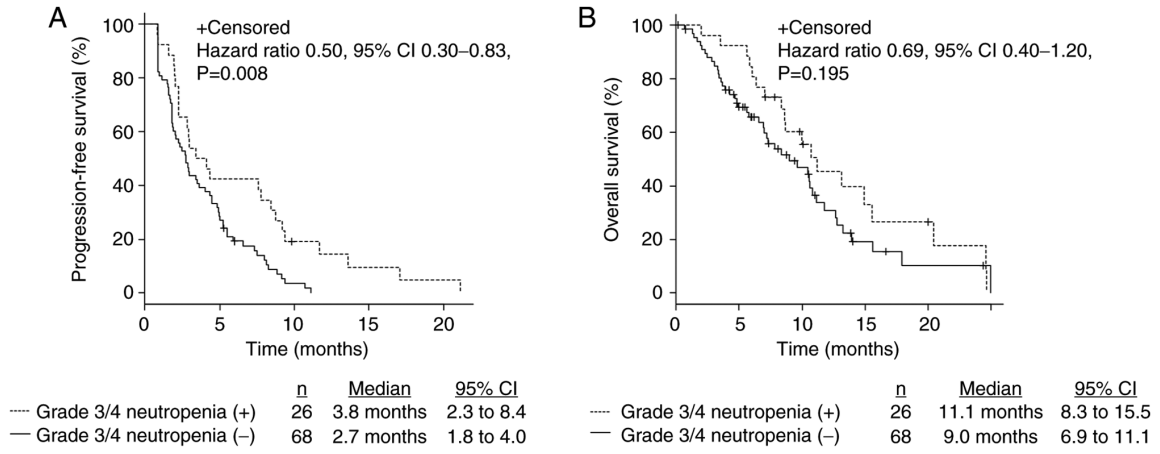

Figure 2. Kaplan-Meier estimates of (A) progression-free survival and (B) overall survival based on presence or absence of grade 3 or 4 neutropenia within the first cycle of treatment. CI, confidence interval.

for patients with mCRC receiving salvage-line therapy is shown in Table VI (11,14,16-20). The clinical outcomes such as PFS, OS, and DCR in patients treated with the FTD/TPI + Bev are better than those in patients treated with the

Table IV. Univariate Cox regression analyses for PFS and OS in patients with metastatic colorectal cancer.

A, PFS

Variable	HR	Lower 95% CI	Upper 95% CI	P-value
Sex (female vs. male)	1.16	0.75	1.79	0.4950
ECOG PS (0 vs. 1 or 2)	1.42	0.89	2.26	0.1340
Age, years (<65 vs. ≥65)	0.91	0.59	1.39	0.6532
Primary tumor location (left vs. right)	0.83	0.52	1.34	0.4615
Liver metastasis (negative vs. positive)	1.53	0.99	2.36	0.0515
Lung metastasis (negative vs. positive)	0.91	0.56	1.47	0.6975
Peritoneal metastasis (negative vs. positive)	1.05	0.68	1.59	0.8289
Lymph node metastasis (negative vs. positive)	2.22	1.40	3.51	0.0006[c]
Tissue *RAS* mutation (negative vs. positive)	0.68	0.44	1.05	0.0826
Time from the start of first-line chemotherapy, months (<18 vs. ≥18)	0.65	0.42	1.02	0.0586
Grade 3 or 4 neutropenia within the first cycle of treatment (negative vs. positive)	0.50	0.30	0.84	0.0080[b]
Treatment regimen (bevacizumab vs. other)	0.78	0.51	1.20	0.2623

B, OS

Variable	HR	Lower 95% CI	Upper 95% CI	P-value
Sex (female vs. male)	1.04	0.62	1.75	0.8573
ECOG PS (0 vs. 1 or 2)	1.83	1.07	3.12	0.0273[a]
Age, years (<65 vs. ≥65)	0.81	0.47	1.37	0.4292
Primary tumor location (left vs. right)	1.01	0.58	1.77	0.9616
Liver metastasis (negative vs. positive)	1.95	1.15	3.30	0.0126[a]
Lung metastasis (negative vs. positive)	0.81	0.46	1.42	0.4567
Peritoneal metastasis (negative vs. positive)	1.11	0.66	1.85	0.6868
Lymph node metastasis (negative vs. positive)	1.16	0.70	1.91	0.5673
Tissue *RAS* mutation (negative vs. positive)	1.13	0.67	1.89	0.6407
Time from the start of first-line chemotherapy, months (<18 vs. ≥18)	0.91	0.54	1.52	0.7077
Grade 3 or 4 neutropenia within the first cycle of treatment (negative vs. positive)	0.70	0.40	1.20	0.1950
Treatment regimen (bevacizumab vs. other)	1.37	0.81	2.33	0.2423

[a]P<0.05; [b]P<0.01; [c]P<0.001. PFS, progression-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group Performance Status; *RAS*, rat sarcoma viral oncogene homolog.

FTD/TPI monotherapy (Tables VI and SIII) (5,6,11,14,16-28). Furthermore, the clinical outcomes of FTD/TPI + Bev of this study were comparable to previous reports. Thus, FTD/TPI + Bev may be a more effective regimen than FTD/TPI monotherapy. On the other hand, the incidence of grade 3 or 4 CIN of FTD/TPI + Bev is higher than FTD/TPI monotherapy (Tables VI and SIII) (5,6,11,14,16-28). CIN was an independent predictive factor for the efficacy of FTD/TPI monotherapy, as reported previously (11,30-32). As mentioned before, pharmacokinetics and pharmacodynamics analysis in the RECOURSE trial suggests a dose-response relationship between FTD exposure and CIN, in agreement with the findings of the dose-escalation studies that a higher rate of CIN at higher doses of FTD/TPI leads to greater efficacy of the drug (10). Furthermore, results of a randomized phase II trial comparing FTD/TPI + Bev with FTD/TPI monotherapy showed that grade 3 or higher neutropenia in the FTD/TPI + Bev group was 67 vs. 38% in the FTD/TPI monotherapy group and PFS in the FTD/TPI + Bev group is significantly longer than that in the FTD/TPI monotherapy group (14). This is because anti-angiogenic drugs can normalize tumor vasculature, alleviate hypoxia, increase drug delivery, and elevate antitumor immune cells; because tumors are accompanied by abnormal vascular structure, tumor interstitial fluid pressure increases owing to vascular leakage, accompanied by hypoxia (33). In addition, a meta-analysis showed that Bev was associated with an increased risk of high-grade neutropenia, as inhibition of the VEGF receptor blocks hematopoietic stem cell cycle, differentiation, and recovery after bone marrow suppression (34). According to the above hypothesis, the

Table V. Multivariate Cox regression analyses for PFS and OS in patients with metastatic colorectal cancer.

A, PFS

Variable	HR	Lower 95% CI	Upper 95% CI	P-value
Liver metastasis (negative vs. positive)	1.82	1.17	2.83	0.0079[b]
Lymph node metastasis (negative vs. positive)	2.23	1.40	3.54	0.0007[c]
Grade 3 or 4 neutropenia within the first cycle of treatment (negative vs. positive)	0.51	0.30	0.86	0.0118[a]

B, OS

Variable	HR	Lower 95% CI	Upper 95% CI	P-value
ECOG PS (0 vs. 1 or 2)	2.26	1.29	3.97	0.0043[b]
Liver metastasis (negative vs. positive)	2.31	1.34	3.98	0.0027[b]

[a]P<0.05; [b]P<0.01; [c]P<0.001. PFS, progression-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group Performance Status.

Table VI. Previous reports of clinical outcomes in patients with metastatic colorectal cancer treated with trifluridine/tipiracil plus bevacizumab as a late-line treatment.

Author, year (ref.)	Number of patients	RR, %	DCR, %	PFS, months	OS, months	Grade 3 or 4 neutropenia in all treatment periods, %
Kuboki *et al*, 2017 (11)	21	0.0	64.0	3.7	11.4	72.0
Kotani *et al*, 2019 (17)	60	5.0	53.3	3.7	8.6	50.0
Matsuhashi *et al*, 2019 (18)	17	0.0	70.1	6.8	14.1	41.2
Pfeiffer *et al*, 2020 (14)	46	2.2	67.4	4.6	9.4	67.4
Fujii *et al*, 2020 (19)	21	0.0	76.1	5.6 (TTF)	14.4	52.4
Shibutani *et al*, 2020 (20)	36	8.3	58.3	-	-	38.9
Nose *et al*, 2020 (16)	32	-	-	4.7	11.7	53.1
Data in present study	94	0.0	44.7	2.9	10.0	51.1

RR, response rate; DCR, disease control rate; PFS, progression-free survival; OS, overall survival; TTF, time to treatment failure; -, not available.

clinical outcome of FTD/TPI + Bev was better than that of FTD/TPI monotherapy. Furthermore, it was similar to the results of several second-line clinical trials (35,36) that investigated a combination therapy consisting of a chemotherapy and anti-VEGF antibody for patients with mCRC. We did not discuss the results of first-line clinical trials in this manuscript. Nevertheless, this study was limited by the relatively small number of patients and its retrospective nature. Despite these limitations, the results of this study provide important and novel insights into the clinical use of FTD/TPI + Bev in salvage-line chemotherapy. For mCRC patients without neutropenia after the initiation of FTD/TPI + Bev, we should consider early image evaluation and treatment changes (e.g., regorafenib) or BSC. In conclusion, the clinical outcomes of FTD/TPI + Bev were comparable to previous reports that showed it to be more effective than FTD/TPI monotherapy. Grade 3 or 4 CIN within the first cycle of treatment is an early predictive marker of the chemotherapeutic efficacy of FTD/TPI + Bev. This could be a useful biomarker for optimizing treatment decisions in daily clinical practice.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

DK, HO and ES conceptualized and designed the present study. DK, HO, ES, AO, TW, KYo, TS, IN, MO, DT, KC and KYa acquired and analyzed the data. DK, HO, ES and AO interpreted the data. DK and HO drafted the initial manuscript and performed statistical analysis. ES, AO, TW, KYo, TS, IN, MO, DT, KC and KYa critically revised the manuscript for important intellectual content. ES and KYa supervised the study. DK, HO and ES confirmed the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Institutional Review Board of the Japanese Foundation for Cancer Research (Tokyo, Japan; approval no. 2020-1017). The protocol is described on the hospital website, and subjects were provided the opportunity to opt-out; therefore, no additional consent was required from patients. All experiments were performed in accordance with the Declaration of Helsinki.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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