


# Phase II trial of biweekly cetuximab and irinotecan as third-line therapy for pretreated *KRAS* exon 2 wild-type colorectal cancer

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Efficacy and safety of biweekly cetuximab plus irinotecan were evaluated to provide guidance for its use in Japan as third-line treatment for pretreated metastatic colorectal cancer (mCRC) patients harboring wild-type *KRAS* exon 2. Objective response rate (ORR) was used as primary endpoint based on an expected proportion of 0.23 with confidence width of 0.298 (95% CI, 0.105-0.403), which showed 35 to be the minimal participant number. Forty patients, refractory to first- and second-line chemotherapy containing irinotecan, oxaliplatin, and fluoropyrimidine, were enrolled. ORR and disease control rate were 25.0% (95% CI: 11.5-38.4) and 72.5% (95% CI: 56.8-86.4), respectively. Median progression-free survival (PFS), overall survival (OS), and number of courses were 5.70 months (95% CI: 2.7-7.9), 15.1 months (95% CI: 11.8-19.0), and 10.5 (range: 3.0-31.0), respectively. Grade 3 adverse events were skin toxicity (12.5%), diarrhea (10.0%), neutropenia (5.0%), febrile neutropenia (5.0%), nausea (5.0%), anorexia (5.0%), and fatigue (2.5%).  $C_{\max}$  mean was 723.2  $\mu\text{g/mL}$  after first dose. High area under the curve (AUC)<sub>last</sub> variance was associated with  $t_{1/2}$  range of 131.2-1209.6 hours (median, 174.4 hours). Early tumor shrinkage (ETS) and median depth of response were 25.0% and 13.0%, respectively. Mutation frequencies in *KRAS* exon 3 or 4, *NRAS*, *BRAF*, and *PIK3CA* were 5.5%, 2.7%, 8.3%, and 5.5%, respectively. Multivariate Cox regression analysis assessed whether any gene mutations and ETS are predictors for PFS, and whether performance status, synchronous metastasis, and ETS are predictors for OS. Importantly, the data provide guidance for a biweekly cetuximab plus irinotecan regimen in mCRC patients.

## KEYWORDS

biweekly cetuximab, *BRAF* mutation, colorectal cancer, early tumor shrinkage, *RAS* mutation

**Abbreviations:** AUC, area under the curve; CapeOX, capecitabine and oxaliplatin; CI, confidence interval; C-mab, cetuximab; CPT-11, irinotecan hydrochloride hydrate; CTCAE, Common Terminology Criteria for Adverse Events; DpR, depth of response; EGFR, epidermal growth factor receptor; ETS, early tumor shrinkage; FOLFIRI, 5-fluorouracil and folinic acid with irinotecan hydrochloride hydrate; FOLFOX, 5-fluorouracil and folinic acid with oxaliplatin; GeoMean, geometric mean; HR, hazard ratio; mCRC, metastatic colorectal cancer; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; PK, pharmacokinetics; PS, performance status.

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## 1 | INTRODUCTION

Cetuximab (C-mab), an IgG1 human/mouse chimera-type monoclonal antibody, prevents dimerization of EGFR by binding to the antigen epitope in EGFR domain 3, which inhibits ligand binding and downstream signaling.<sup>1</sup> Furthermore, it stimulates receptor internalization and degradation that may trigger an antitumor antibody-dependent cell-mediated cytotoxicity reaction.<sup>2</sup>

C-mab was approved for mCRC treatment in Japan in July 2008 to be given as an initial i.v. infusion of 400 mg/m<sup>2</sup> on day 1 infused over 120 minutes followed by weekly doses of 250 mg/m<sup>2</sup> infused over 60 minutes.<sup>3</sup> In the National Comprehensive Cancer Network (NCCN) Guidelines<sup>®</sup> version 2.2017 Colon Cancer, both weekly and biweekly C-mab are indicated in combination with a CPT-11-based therapy or as a single-agent therapy in *KRAS/NRAS* WT patients if C-mab or panitumumab was not used as initial therapy. Typically, C-mab and CPT-11 are used in combination as a third-line treatment for mCRC because the efficacy of the combination therapy is higher than that of C-mab monotherapy,<sup>4</sup> and CPT-11 is given every 2 weeks. Therefore, if similar efficacy and safety could be achieved with a biweekly dose of C-mab, it would be more convenient for both the patient and the care provider. However, there are few reports about the efficacy and safety of biweekly C-mab plus CPT-11 against mCRC in Japan.<sup>5</sup>

Recently, there have been several reports about predictive parameters for monitoring treatment efficacy, specifically in mCRC patients receiving anti-EGFR therapy. ETS is the decrease in tumor load at the time of first imaging after the start of treatment.<sup>6</sup> It is an early indicator of sensitivity to treatment. DpR is defined as the percentage of tumor shrinkage based on the longest diameter or reconstructed volume at the smallest observed volume compared with baseline and reflects OS.<sup>6</sup> In contrast, *KRAS* exons 2, 3, and 4/*NRAS* exons 2, 3, and 4/*BRAF/PIK3CA* mutations are reported as negative predictors of the efficacy of anti-EGFR therapy.<sup>7,8</sup> However, there are few reports about the effect of third-line chemotherapy on ETS/DpR or the molecular status of the EGFR pathway.

The aim of this phase II trial was to evaluate the efficacy and safety of biweekly C-mab plus CPT-11 as third-line treatment in patients with pretreated *KRAS* exon 2 WT mCRC in Japan.

## 2 | MATERIALS AND METHODS

### 2.1 | Patients

Patients were enrolled in the present study if they met the following criteria: (i) histopathologically proven colorectal adenocarcinoma with *KRAS* exon 2 WT according to *KRAS* status test carried out in our institution using Luminex technology (MEBGEN *KRAS* Mutation Detection Kit; MBL, Tokyo, Japan); (ii) ECOG PS of 0-2; (iii) presence of measurable metastatic disease as defined by the RECIST criteria; (iv) presence of radiographically confirmed disease progression during previous chemotherapy with CPT-11 or within 3 months after the last chemotherapy dose; (v) treatment failure (defined as disease progression/discontinuation as a result of toxicity) of fluoropyrimidine- and

oxaliplatin-based chemotherapy; (vi) adequate bone marrow reserve (neutrophil count: >1500/mm<sup>3</sup>, platelet count: >100 000/mm<sup>3</sup>); (vii) adequate hepatic function defined by aspartate aminotransferase and alanine aminotransferase levels of <100 U/L (<200 U/L in patients with liver metastases) and a total bilirubin level of <1.5 mg/dL; and (viii) adequate renal function defined by a serum creatinine level of <1.5 mg/dL. Exclusion criteria were as follows: (i) uncontrollable ascites or pleural effusion; and (ii) serious comorbidities, such as pulmonary fibrosis or interstitial pneumonia, uncontrollable diabetes mellitus, severe heart disease, other active malignancy, active inflammation, or other serious medical conditions. This study was approved by our institutional review board (IRB number: 2010-1121) and conducted in accordance with the protocol and in compliance with the Declaration of Helsinki. The study was registered with the University Hospital Medical Information Network (UMIN) Clinical Trials Registry, number 000019893. Written informed consent was obtained from each patient prior to treatment.

### 2.2 | Treatment schedule and assessment

C-mab was initially given at a dose of 500 mg/m<sup>2</sup> as a 2-hour infusion followed by biweekly dose of 500 mg/m<sup>2</sup> as a 1-hour infusion. CPT-11 was given at a dose of 150 mg/m<sup>2</sup> biweekly. Patients were premedicated with an antihistamine (eg, 10 mg chlorpheniramine maleate i.v.) to minimize the risk of infusion-related reactions associated with C-mab. Toxicity was graded according to the National Cancer Institute (Common Terminology Criteria for Adverse Events [CTCAE], version 4.0).

Grade 3 febrile neutropenia was defined by an absolute neutrophil count <1000/mm<sup>3</sup> associated with a single temperature of >38.3°C or a sustained temperature of ≥38°C for more than 1 hour; grade 4 was defined by life-threatening consequences, which required urgent intervention according to the CTCAE version 4.0.

Grade 2 hypersensitivity necessitated C-mab discontinuation; therefore, the infusion was slowed to 50% of the prior infusion rate given in cases of grade 1 allergic/hypersensitivity reactions. Grades 3-4 hypersensitivity also necessitated discontinuation of C-mab. C-mab dosage was withheld in cases of grade 3 skin toxicity until the severity decreased to ≤grade 2 and resumed without dose modification. If grade 3 skin toxicity reoccurred during resumed C-mab dosage, it was completely withheld until the severity decreased to ≤grade 2 and, then, C-mab dosage was resumed at a dose reduced by 100 mg/m<sup>2</sup>. A pre-emptive skin treatment regimen was not determined by the protocol; however, the recommendations are as follows: initiated 1 day before giving the first cetuximab dose, day 1, and continued through weeks 1 to 6. It consisted of skin moisturizer applied to the face, hands, feet, neck, back, and chest daily in the morning; sunscreen applied to exposed skin areas before going outdoors; topical steroid applied to face, hands, feet, neck, back, and chest daily at bedtime; and minocycline 100 mg per day. Dose modifications and treatment alterations were also carried out in cases of CPT-11-associated toxicity. In cases of grade 4 thrombocytopenia or grades 3-4 nonhematological toxicity, CPT-11 was discontinued. The CPT-11 dose was reduced by 30 mg/m<sup>2</sup> in cases of grade 4 neutropenia, grades 2-3 thrombocytopenia or grade 3 nonhematological toxicity. Other dose adjustments were made on an individual patient basis.

Treatment was discontinued if the tumor progressed or severe toxicity developed, or at the patient's request. There was no set maximum number of courses.

### 2.3 | C-mab pharmacokinetics analysis

Blood samples were obtained from 12 patients for analysis of serum C-mab concentration before and at 2 hours (end of the first infusion); 24 hours after dose of C-mab on day 1; day 7 (168 hours after the first dose); and then before dosage on days 15 (366 hours), 29 (732 hours), 43 (1008 hours), and 57 (1344 hours). Descriptive statistics of serum C-mab concentration were calculated and summarized in a concentration-time curve by R (version 3.3.0). PK parameters were estimated based on the C-mab concentration data and actual sampling time during the first dose interval by standard non-compartmental analysis using PKNCA package<sup>9</sup> (version 0.8.1) run through R and then summarized by descriptive statistics.

### 2.4 | Extended RAS, PIK3CA, and BRAF mutation analysis

Genomic DNA was extracted from formalin-fixed paraffin-embedded tissue obtained from biopsies or surgical resections. *KRAS* codon 61 (Q61K, Q61E, Q61L, Q61P, Q61R, and Q61H); *KRAS* codon 146 (A146T, A146S, A146P, A146E, A146V, and A146G); *BRAF* codon 600 (V600E); *NRAS* codon 12 (G12S, G12C, G12R, G12D, G12V, and G12A), codon 13 (G13S, G13C, G13R, G13D, G13V, and G13A), and codon 61 (Q61K, Q61E, Q61L, Q61P, Q61R, and Q61H); and *PIK3CA* exon 9 codon 542 (E542K), codon 545 (E545K), codon 546 (E546K), and exon 20 codon 1047 (H1047R and H1047L) were analyzed using Luminex (xMAP) technology (GENOSEARCH Mu-PACK; MBL). Sensitivity of these assays was reported to be 1% according to manufacturer's instructions.

### 2.5 | Statistical analysis

This study was designed as a phase II trial to evaluate the efficacy and safety of biweekly C-mab plus CPT-11 dosage. Primary endpoint was ORR. Other evaluated parameters were PFS, OS, adverse events according to CTCAE version 4.0, and pharmacokinetic parameters representing secondary endpoints. Our statistical design and hypotheses are as follows. Pfeiffer et al reported an ORR of 25.7% for cetuximab (500 mg/m<sup>2</sup>, biweekly dosing) plus CPT-11 in advanced/recurrent colorectal cancer patients.<sup>10</sup> In addition, Martín-Martorel et al reported an ORR of 22.5% using combination therapy.<sup>11</sup> Thus, we predicted an ORR range of approximately 23.0%-26.0% for the combination therapy with biweekly cetuximab plus CPT-11. Therefore, the expected proportion was estimated to be 0.23 with a confidence width of 0.298 and the confidence level was set as 95%. Using these parameters, the calculated minimal sample size was 31 to have 95% CI of 0.105-0.403. Considering a 10% dropout rate, 35 patients were needed for the present study. Tumor response was assessed objectively once every 2 months after each course according to RECIST (ver. 1.1), and the

ORR was considered indicative of the antitumor effect. All data were obtained by reviewing medical records and/or imaging. We confirmed age, gender, PS, primary location, pathology of the primary tumor, metastatic sites, and synchronous metastasis. Primary tumors originating in the splenic flexure, descending colon, sigmoid colon, or rectum were classified as left-sided mCRC. Primary tumors originating in the cecum, ascending colon, hepatic flexure, or transverse colon were classified as right-sided mCRC. Post-hoc analyses were carried out for ETS, DpR, and molecular status of the EGFR signaling pathway. PFS and OS were estimated by the Kaplan-Meier method and the log-rank test. PFS was defined as the time from the first day of treatment to either the first objective evidence of disease progression or to death from any cause. OS was defined as the time from the first day of treatment to death from any cause. ETS was defined as a relative change in the sum of the longest diameters at week 8 compared to that at baseline (cutoff: 20.0%). DpR was defined as the relative change in the sum of the longest diameters at the nadir compared to that at baseline (cutoff: median). We adopted both definitions according to previous studies of mCRC. All reported *P*-values were the result of 2-sided tests, with *P* < .05 considered statistically significant.

Prognostic factors showing *P* < .2 in the univariate analysis were included in the multivariate analysis. Correlations between ETS/DpR and clinical outcomes were estimated using Pearson's correlation coefficient. All statistical analyses were carried out with EZR (Saitama Medical Center, Jichi Medical University), which is a graphical user interface for R (The R Foundation for Statistical Computing).

## 3 | RESULTS

### 3.1 | Patient characteristics

A total of 40 patients were enrolled between October 2011 and November 2014 (Table 1); the data cutoff period was August 2015. Thirty-four patients had a PS of 0, whereas 6 had a PS of 1. All patients had *KRAS* exon 2 WT mCRC. Prior oxaliplatin-containing regimens included CapeOX in 11 patients and FOLFOX (infusion and bolus 5-fluorouracil with oxaliplatin) in 29 patients; prior CPT-11-containing regimens included FOLFIRI (infusion and bolus 5-fluorouracil with CPT-11) in 6 patients. Thirty-four patients received both oxaliplatin-based therapy and CPT-11-based therapy. Six patients received CPT-11-based therapy only because of a relapse after, during, or within 6 months after adjuvant chemotherapy. In addition, a modified FOLFOX-6 regimen was given as adjuvant chemotherapy. Thirty-three patients had received bevacizumab treatment prior to study entry. All patients discontinued prior CPT-11-based chemotherapy as a result of disease progression. The previous oxaliplatin-based regimen was discontinued as a result of disease progression in 30 patients and toxicity in 10 patients (neuropathy in 8 patients and allergy in 2 patients).

### 3.2 | Treatment results

Median number of C-mab and CPT-11 doses was 10.5 (range, 3.0-35.0) and 9.5 (range, 2.0-35.0), respectively. Four patients required

**TABLE 1** Characteristics of patients with pretreated *KRAS* exon 2 WT mCRC in Japan

Clinical variable	N = 40
Median age, years (range)	59 (31-72)
Gender, n (%)	
Male	14 (35.0)
Female	26 (65.0)
ECOG PS, n (%)	
0	34 (85.0)
1	6 (15.0)
Site of the primary tumor, n (%)	
Right	8 (20.0)
Left	32 (80.0)
Synchronous metastases, n (%)	24 (60.0)
Metastasis sites, n (%)	
Liver	28 (70.0)
Lung	17 (42.5)
Lymph node	13 (32.5)
Other	18 (45.0)
Metastatic disease at multiple sites, n (%)	31 (77.5)
Histology, n (%)	
Differentiated	36 (90.0)
Undifferentiated	4 (10.0)

mCRC, metastatic colorectal cancer; PS, performance status.

C-mab dose reductions because of skin toxicities. Furthermore, 12 patients required CPT-11 dose reductions, primarily as a result of neutropenia and gastrointestinal toxicity. Protocol treatment was discontinued in 40 patients as a result of disease progression (N = 38) and skin toxicities (N = 2). There were no instances of withdrawal as a result of patient request. Dose intensity of C-mab and CPT-11 was 97.8% and 91.9%, respectively.

### 3.3 | Efficacy

Median follow-up time was 15.1 months (95% CI, 11.8-19.0). Among the 40 patients, no patient achieved a complete response; 10 patients experienced a confirmed partial response; and 19 showed stable disease. Ten patients had progressive disease, and treatment response could not be evaluated in 1 patient because of symptomatic deterioration prior to a radiological response evaluation. The ORR rate, which was the primary endpoint, was 25% (95% CI, 11.5-38.4). Disease control rate (complete response, partial response, or stable disease) was 72.5% (95% CI, 56.8-86.4). Median PFS was 5.7 months (95% CI, 2.7-7.9), and median OS was 15.1 months (95% CI, 11.8-19.0) (Figure 1).

### 3.4 | Toxicity

Grade 3 neutropenia was observed in 2 patients (5.0%) (Table 2). Febrile neutropenia was observed in 2 patients (5.0%), which was successfully managed by treatment with granulocyte-colony stimulating factor

and antibiotics. Skin toxicity, including acne, rash, dry skin, pruritus, acne-form dermatitis, and papular rash, was observed in 35 patients (87.5%). Five patients (12.5%) experienced grade 3 skin toxicity (acne-form rash: N = 2, dry skin: N = 1, nail disorder: N = 2). Other grade 3 nonhematological toxicities included diarrhea (10.0%), nausea (5.0%), anorexia (5.0%), and fatigue (2.5%). Five patients (15.0%) experienced grade 1/2 allergic reactions. No grade 4-5 toxicities were noted.

### 3.5 | Pharmacokinetic analysis

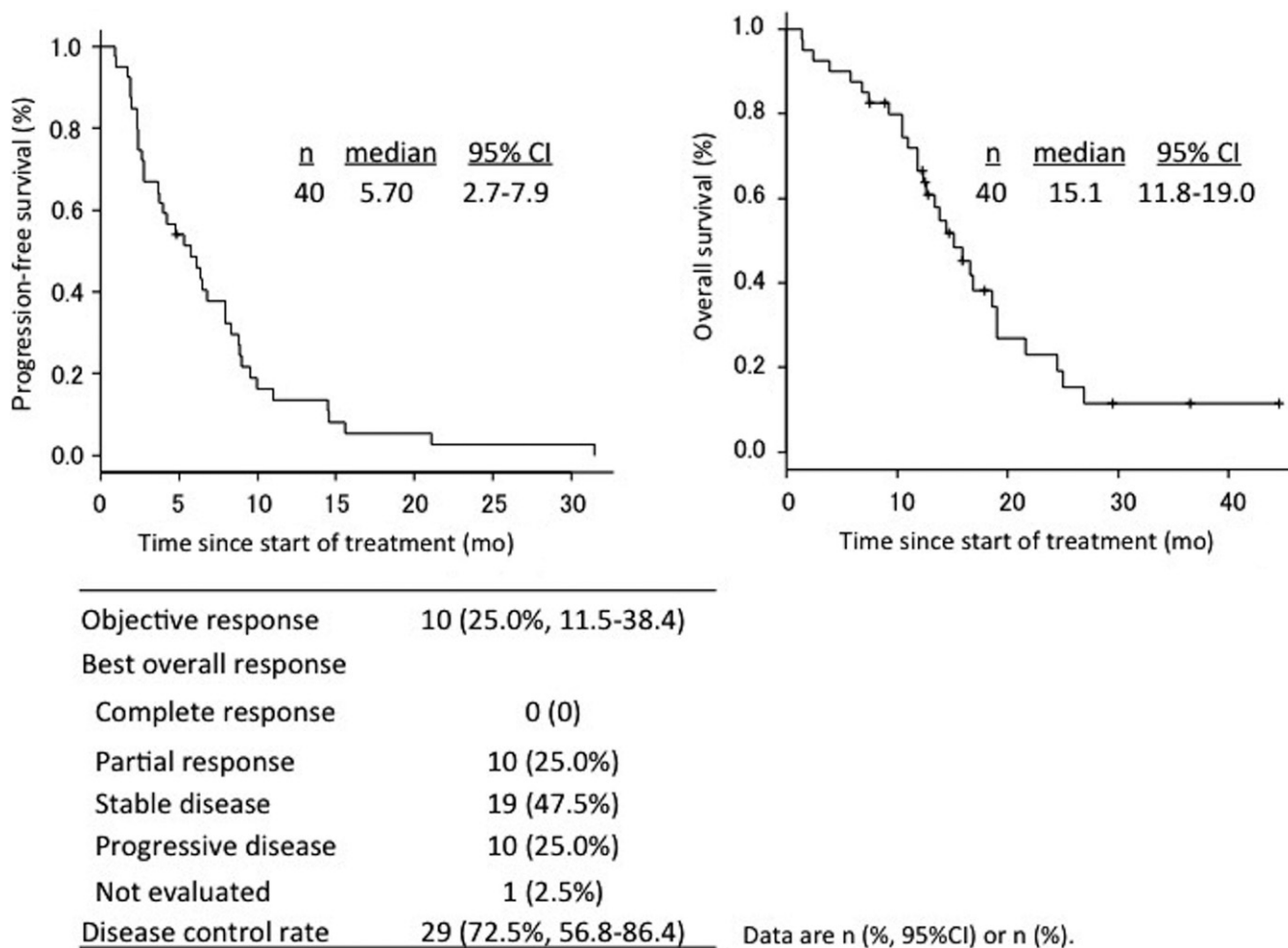
The  $C_{max}$  geometric mean (GeoMean) of C-mab was 723.2  $\mu\text{g/mL}$  after the first dose (Figure 2, Table 3). The high variance in  $AUC_{last}$  was in conjunction with the  $t_{1/2}$  of 174.4 hours with the median variance ranging from 131.2 to 1209.6 hours (Table 3). The trough GeoMean concentration of C-mab tended to increase during the first 3 dosages before reaching a steady state; thereafter, a large interindividual variability was observed in this parameter. This observation was in line with the  $t_{1/2}$  of C-mab in the present study and a previously estimated mean value (approximately 130-160 hours) in another study using a biweekly regimen.<sup>5</sup>

### 3.6 | Post-hoc analyses

Genetic profiling (N = 36) showed that the frequencies of *KRAS* exon 3 or 4, *NRAS*, *BRAF*, and *PIK3CA* mutations were 5.5% (2/36), 2.7% (1/36), 8.3% (3/36), and 5.5% (2/36), respectively. (Figure 3) No co-mutations were observed in these mutations. Response rates of *KRAS*/*NRAS* WT, *KRAS*/*NRAS*/*BRAF* WT, and *KRAS*/*NRAS*/*BRAF*/*PIK3CA* WT (all WT) were 27.3% (9/33), 30.0% (9/30), and 32.1% (9/28), respectively. (Figures 3 and S1) Both PFS and OS were significantly longer in the *KRAS*/*NRAS*/*BRAF* WT group than in the *KRAS*/*NRAS*/*BRAF* mutant group (PFS: 6.3 vs 1.8 months,  $P < .0001$ ; OS: 15.9 vs 7.2 months,  $P < .0001$ ). PFS in the group of all WT was also significantly longer than those in the group of any gene mutation; however, OS was not significantly different between the 2 groups (PFS: 6.3 vs 2.1 months,  $P = .04$ ; OS: 15.1 vs 10.4 months,  $P = .60$ ). The ETS rate was 25%, and median DpR was 13% (range: -77% to 77%). Compared to ETS <20%, ETS  $\geq$ 20% was associated with significantly prolonged OS and PFS. Further, compared to DpR <13%, DpR  $\geq$ 13% (median value) was associated with significantly prolonged OS and PFS. (Figure 4) There was a moderate positive correlation between DpR and clinical outcomes (OS:  $r = .52$ , PFS:  $r = .49$ ,  $P < .05$ ). The ETS rate and median DpR were higher in the *KRAS*/*NRAS*/*BRAF* WT group than in the *KRAS*/*NRAS*/*BRAF* mutant group (ETS: 26.6% vs 0%; DpR: 15.0% vs -28.0%) (Figure S1).

### 3.7 | Multivariate analysis

A multivariate Cox regression analysis was conducted to assess whether the appearance of any gene mutation and ETS are predictors for PFS (any gene mutation: HR 5.20, 95% CI 1.30-20.9,  $P = .02$ ; ETS: HR 0.21, 95% CI 0.05-0.84,  $P = .02$ ) and, similarly, whether PS, synchronous metastasis, and ETS are predictors for OS



**FIGURE 1** Response rate, progression-free survival, and overall survival in all patients

(PS: HR 4.61, 95% CI 1.28-16.5,  $P = .02$ ; synchronous metastasis: HR 2.60, 95% CI 1.08-6.30,  $P = .03$ ; ETS: HR 0.17, 95% CI 0.05-0.56,  $P = .003$ ) (Table 4).

## 4 | DISCUSSION

The aim of the present study was to evaluate the efficacy and safety of biweekly C-mab plus CPT-11 dosage as the third-line treatment in patients with pretreated *KRAS* exon 2 WT mCRC in Japan. We also evaluated the relationship between ETS/DpR, or the molecular status of the EGFR signaling pathway, and the efficacy of anti-EGFR antibody therapy. Both efficacy and safety in this study were similar to those reported previously. Furthermore, the ETS/DpR and the molecular status of the EGFR signaling pathway were associated with clinical outcomes in third-line biweekly C-mab plus CPT-11 therapy.

Shitara et al reported the first Japanese study on biweekly C-mab and CPT-11 as third-line treatment for *KRAS* exon 2 WT mCRC that found a partial response in 9 among 30 Japanese patients who received this treatment. ORR was 30.0% (95% CI, 14.7-49.4), and the disease control rate was 76.7% (95% CI, 57.7-90.0). Median PFS was 5.30 months, and median OS was

10.8 months. Grade 3 skin toxicity was observed in 3 patients (10.0%). Mean  $C_{max}$  was 195.2  $\mu\text{g/mL}$  on day 1 and 230.8  $\mu\text{g/mL}$  on day 15, and the mean trough concentration was 22.14  $\mu\text{g/mL}$  on day 15 and 38.34  $\mu\text{g/mL}$  on day 29.<sup>5</sup> Pfeiffer et al reported a response rate of 25% in European patients who were treated with biweekly C-mab and CPT-11 as the third-line treatment without genotyping of *KRAS* exon 2. Median PFS and OS were 5.4 months and 8.9 months, respectively, and were comparable to historical controls receiving weekly C-mab and CPT-11 therapy. Grades 3-4 toxicity was rare (diarrhea 10.0%, neutropenia 9.0%, skin 7.0%, nail 3.0%, and fatigue 3.0%). One patient developed a severe allergic reaction.<sup>10</sup> Tabernero et al reported 2 treatment regimens, 500  $\text{mg/m}^2$  biweekly and 250  $\text{mg/m}^2$  weekly dosing schedule, resulting in similar PK data, and AUC of 35 794  $\mu\text{g/mL} \times \text{h}$  vs AUC of 35 574  $\mu\text{g/mL} \times \text{h}$ , respectively.<sup>12</sup> In this present study, both safety and efficacy were similar to those reported previously.

A comparison with historical PK data indicated that exposure to C-mab was higher in our study than in the previous reports.<sup>5,12</sup> Regardless of differences in serum C-mab concentration measurements, quantitative and qualitative assessment results of safety and efficacy were comparable among studies using a biweekly regimen. However, the apparent differences in blood concentration



measurements might suggest a limitation of the interstudy comparison of PK, because the differences could be due to the variations in analytical methods including commercially available ELISA assay kits. Nevertheless, PK profile data of C-mab in our study, such as the accumulating tendency observed, were comparable with previous data.<sup>12</sup>

Early tumor shrinkage and DpR are associated with long-term outcomes in patients with chemorefractory mCRC receiving anti-EGFR antibody in the first- and second-line treatments.<sup>6,13-15</sup> Petrelli et al reported a systematic review and meta-analysis of 21 trials from 10 publications to evaluate the prognostic value of ETS in CRC in relation to OS and PFS.<sup>16</sup> Overall, patients with ETS had better OS (HR, 0.58; 95% CI, 0.53 to 0.64;  $P < .00001$ ) and

PFS (HR, 0.57; 95% CI, 0.47-0.69;  $P < .00001$ ) than patients without ETS. Thus, it is important to evaluate ETS/DpR in patients receiving third-line chemotherapy as well as first- and second-line treatment, although there are only a few reports published on this subject.<sup>17</sup>

Many reports showed the significance of the RAS mutation status on the efficacy of treatment with anti-EGFR antibody and chemotherapy. Additional RAS mutations and other mutations related with the EGFR signaling pathway, such as KRAS exon 3 or 4, NRAS, PIK3CA, and BRAF mutations, predicted a lack of benefit of anti-EGFR antibody treatment in patients who had already received treatment with an anti-EGFR antibody and chemotherapy as first- or second-line therapy.<sup>18-21</sup> Currently, RAS testing for patients with mCRC is recommended by various guidelines, mostly based on the evidence of first-line therapy. However, there were few previous reports about the influence of the RAS mutation and these mutation statuses on the treatment effect in a prospective study of patients previously treated with third-line chemotherapy. In this study, not only KRAS/NRAS WT but also KRAS/NRAS/BRAF WT and all WT

**TABLE 2** Toxicity in patients treated with biweekly C-mab plus CPT-11 as third-line treatment in patients with pretreated KRAS exon 2 WT mCRC in Japan

	Grade 1	Grade 2	Grade 3	Grade 4
<b>Gastrointestinal</b>				
Diarrhea (%)	17 (42.5)	4 (10.0)	4 (10.0)	0 (0)
Nausea (%)	12 (30.0)	5 (12.5)	2 (5.0)	0 (0)
Vomiting (%)	3 (7.5)	0 (0)	0 (0)	0 (0)
Constipation (%)	17 (42.5)	3 (7.5)	0 (0)	0 (0)
<b>Dermatological</b>				
Acne-form rash (%)	11 (27.5)	24 (60.0)	2 (5.0)	0 (0)
Dry skin (%)	19 (47.5)	12 (30.0)	1 (2.5)	0 (0)
Nail disorder (%)	9 (22.5)	5 (12.5)	2 (5.0)	0 (0)
<b>Metabolic disorders</b>				
Fatigue (%)	7 (17.5)	3 (7.5)	1 (2.5)	0 (0)
Anorexia (%)	7 (17.5)	6 (15.0)	2 (5.0)	0 (0)
<b>Hematological</b>				
Neutropenia (%)	25 (62.5)	5 (12.5)	2 (5.0)	0 (0)
Febrile neutropenia (%)	—	—	2 (5.0)	0 (0)
Hypomagnesemia (%)	1 (2.5)	8 (20.0)	0 (0)	0 (0)
Anaphylactic reaction (%)	1 (2.5)	4 (10.0)	0 (0)	0 (0)

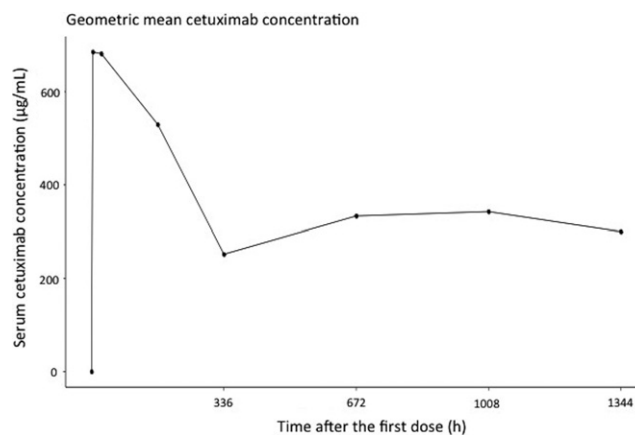
C-mab, cetuximab; CPT-11, irinotecan hydrochloride hydrate; mCRC, metastatic colorectal cancer; —, Not applicable.

**TABLE 3** Pharmacokinetic parameters of cetuximab during the first dosing interval

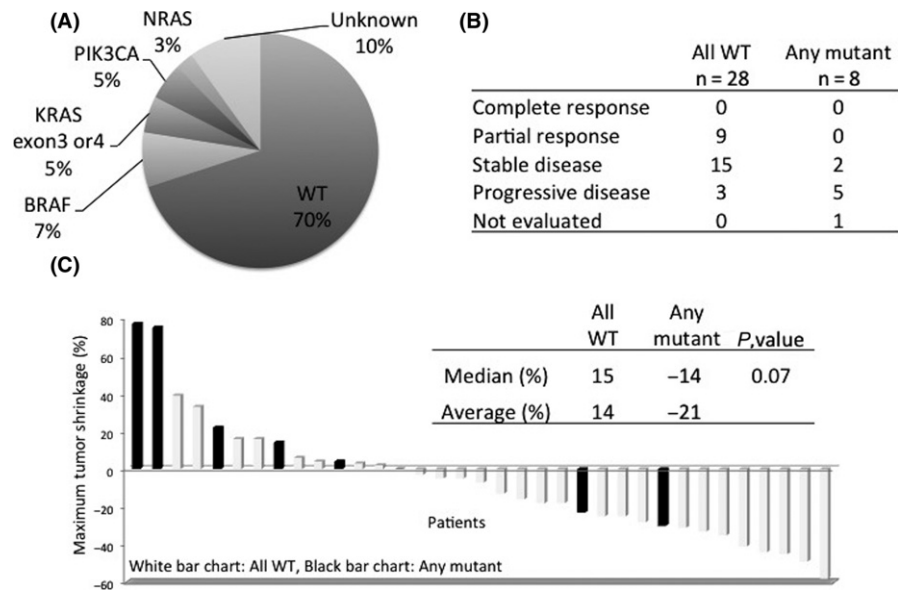
	N	Mean	%CV	Median	Min	Max	GeoMean	%GeoCV
$C_{max}$ ( $\mu\text{g/mL}$ )	12	723.6	3.6	736.8	661.6	745.8	723.2	3.7
$AUC_{last}$ ( $\mu\text{g/mL} \times \text{h}$ )	12	172612	22.0	172607	108214	232344	168620	23.0
$t_{1/2}$ (h)	8	299.6	123.0	174.4	131.2	1209.6	214.8	81.5
	N	Median	Min	Max				
$t_{max}$ (h)	12	2.51	0.92	24.3				

$AUC_{last}$  was calculated by the linear-up log-down interpolation method.  $t_{1/2}$  was estimated only in the patients for whom data were available for at least 3 time points excluding  $t_{max}$  during the terminal elimination phase.

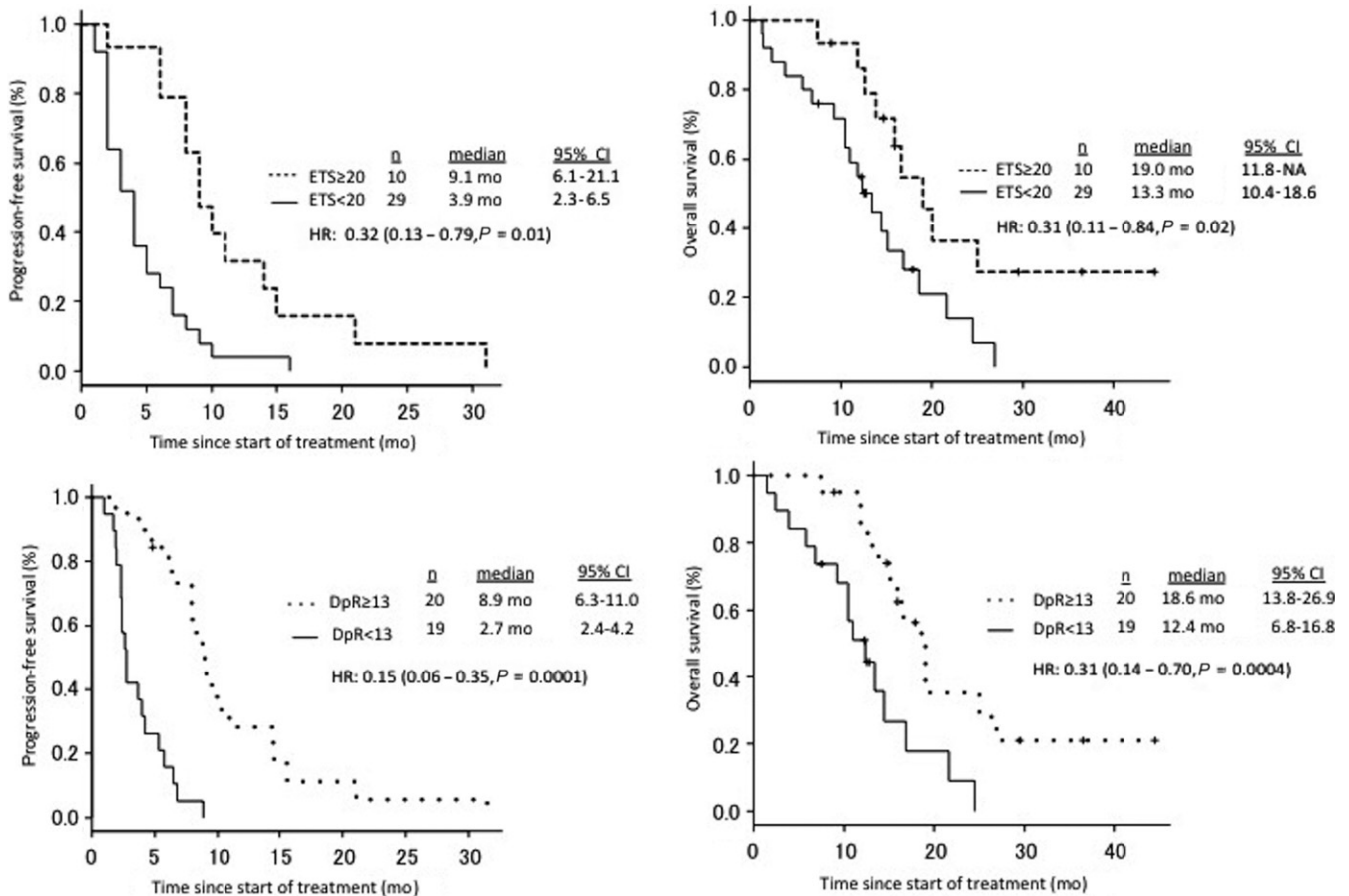
$AUC_{last}$ , area under the curve from zero to the time of the last quantifiable concentration;  $C_{max}$ , maximum observed concentration; CV, coefficient of variation; GeoCV, geometric CV; GeoMean, geometric mean;  $t_{1/2}$ , terminal elimination half-life;  $t_{max}$ , time of occurrence of  $C_{max}$ .



**FIGURE 2** Geometric mean cetuximab concentration. Sample size was 12 except for 168 hours (N = 10, as a result of 2 missing samples), 1008 hours (N = 11, as a result of a value not associated with previous dosage), and 1344 hours (N = 9, as a result of a value below the lower limit of quantification and 2 samples taken at time points deviating from the planned schedule)



**FIGURE 3** Monitoring parameters. A, Frequencies of mutations in the epidermal growth factor receptor (EGFR) pathway. B, the relationship between the molecular status of the EGFR pathway and tumor response; and C, waterfall plot analysis of maximum tumor shrinkage



**FIGURE 4** Relationship between early tumor shrinkage, depth of response, and clinical outcomes

were associated with prolongation of PFS. In Japan, RAS testing is now covered by the national health insurance; however, our study was conducted before this testing was covered.

Previous studies have reported that the primary tumor location in mCRC has an impact on the prognosis and C-mab efficacy. In

several first-line clinical trials, the prognosis was worse for patients with right-sided primary tumors than left-sided primary tumors and patients with RAS WT left-sided tumor had a significantly greater survival benefit from anti-EGFR treatment than from standard chemotherapy or anti-VEGF treatment when added to standard

**TABLE 4** Univariate and multivariate Cox proportional hazards model for progression-free survival (PFS) and overall survival (OS)

PFS	HR	Lower 95% CI	Upper 95% CI	P-value	HR	Lower 95% CI	Upper 95% CI	P-value
Gender (male or female <sup>a</sup> )	0.67	0.34	1.34	.26				
Age (<65 <sup>a</sup> or ≥65) years	0.66	0.30	1.48	.31				
PS (0 <sup>a</sup> or 1)	2.10	0.78	5.66	.13	4.08	0.64	8.15	.19
Primary location (right or left <sup>a</sup> )	1.90	0.84	4.30	.12	1.45	0.30	6.95	.64
Pathology (well, mod or por, sig, muc <sup>a</sup> )	0.45	0.13	1.59	.21				
Synchronous metastasis (yes or no <sup>a</sup> )	2.20	1.08	4.80	.03	2.88	0.88	8.92	.08
Liver metastasis (yes or no <sup>a</sup> )	1.85	0.88	3.87	.10	1.32	0.50	3.49	.57
Lymph node metastasis (yes or no <sup>a</sup> )	0.64	0.32	1.29	.22				
Lung metastasis (yes or no <sup>a</sup> )	0.69	0.35	1.35	.28				
Any gene mutation (yes or no <sup>a</sup> )	2.30	0.99	5.30	.051	5.2	1.30	20.9	.02
ETS (<20 <sup>a</sup> or ≥20)	0.32	0.13	0.79	.01	0.21	0.05	0.84	.02
OS	HR	Lower 95% CI	Upper 95% CI	P-value	HR	Lower 95% CI	Upper 95% CI	P-value
Gender (male or female <sup>a</sup> )	0.64	0.28	1.32	.20	0.58	0.24	1.40	.23
Age (<65 <sup>a</sup> or ≥65) years	1.02	0.45	2.30	.94				
PS (0 <sup>a</sup> or 1)	2.10	0.81	5.80	.12	4.61	1.28	16.5	.02
Primary location (right or left <sup>a</sup> )	1.28	0.52	3.18	.58				
Pathology (well, mod or por, sig, muc <sup>a</sup> )	0.73	0.25	2.10	.58				
Synchronous metastasis (yes or no <sup>a</sup> )	2.10	0.95	4.66	.06	2.60	1.08	6.30	.03
Liver metastasis (yes or no <sup>a</sup> )	1.75	0.76	4.02	.18	2.20	0.89	5.70	.08
Lymph node metastasis (yes or no <sup>a</sup> )	0.98	0.45	2.12	.97				
Lung metastasis (yes or no <sup>a</sup> )	0.55	0.25	1.20	.13	0.68	0.28	1.60	.38
Any gene mutation (yes or no <sup>a</sup> )	1.25	0.52	3.0	.60				
ETS (<20 <sup>a</sup> or ≥20)	0.31	0.11	0.84	.02	0.17	0.05	0.56	.003

<sup>a</sup>Reference.

CI, confidence interval; ETS, early tumor shrinkage; HR, hazard ratio; mod, moderately differentiated adenocarcinoma; muc, mucinous adenocarcinoma; OS, overall survival; PFS, progression-free survival; por, poorly differentiated adenocarcinoma; PS, performance status; sig, signet ring cell adenocarcinoma; well, well-differentiated adenocarcinoma.

chemotherapy.<sup>22</sup> In this present study and in earlier reports, patients with right-sided tumor had shorter PFS than patients with left-sided tumor (HR 1.90 [0.84-4.30] in univariate analysis), although we did not observe a significant difference, which could be due to the small sample size. However, a significant difference might be observed if the study is repeated with a larger number of subjects. In contrast, the difference tended to be small in *KRAS/NRAS/BRAF* WT patients (HR 1.45 [0.52-4.0] in univariate analysis) (Table S1). Thus, CPT-11 plus biweekly cetuximab might be an alternative treatment option for *KRAS/NRAS/BRAF* WT patients with right-sided mCRC.

The present study has some limitations. Although *RAS* testing was covered by insurance from 2015 in Japan, we could not carry out *RAS* testing on all patients during the trial term. The sample size was small for evaluating the relationship between each gene mutation and the efficacy of anti-EGFR treatment.

Importantly, the efficacy and safety of biweekly C-mab and CPT-11 combination therapy were similar in mCRC patients from Japan and western populations, demonstrating that the biweekly C-mab

and CPT-11 course is an alternative to weekly regimens. ETS/DpR is a potential parameter for monitoring treatment efficacy. In addition, it may be beneficial for patients undergoing third-line treatment to monitor mutations linked to the EGFR signaling pathway, such as *KRAS* exon 3 or 4, *NRAS*, *BRAF*, and *PIK3CA*.

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#### CONFLICTS OF INTEREST

E Shinozaki received honoraria from speaker's bureau, Taiho Pharmaceutical Co., Ltd, and Eli Lilly and Co., Ltd. All remaining authors declared no conflicts of interest for this article.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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