A multicenter single-arm Phase II clinical trial of second-line FOLFIRI plus panitumumab after first-line treatment with FOLFOX plus panitumumab for initial *RAS* wild-type colorectal cancer with evaluation of circulating tumor DNA: A protocol study

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Abstract. The efficacy and safety of the continuous use of panitumumab in first- and second-line treatments for colorectal cancer (CRC) have yet to be determined. Liquid biopsy of circulating tumor DNA is capable of assessing the gene mutation status at several time-points, and could predict the efficacy of ongoing panitumumab treatment. To address these two points, a multicenter single-arm Phase II clinical trial will be conducted by evaluating the effect of FOLFIRI with panitumumab as second-line chemotherapy in patients with CRC, after failure or intolerance of first-line treatment with FOLFOX with panitumumab. The primary endpoint is the 6-month progression-free survival rate. Gene mutation status using circulating tumor DNA will be assessed at multiple time-points during the study period as one of the secondary endpoints. The observed 6-month PFS rate will be compared with the threshold 6-month PFS rate of 35% with a one-sided significance level of 10% using the binomial exact test. The target number of cases in this study is 55 patients. The study protocol was approved by the Institutional Review Board of the Epidemiological and Clinical Research Information Network (17-0601-1) and will be conducted after approval by the Institutional Review Board of each participating institute. This study is registered in UMIN (UMIN000026817, March 31, 2017). The results of the present study will be presented at related international congresses, and will be disseminated in peer-reviewed journals.

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Panitumumab therapy with FOLFILI after panitumumab with FOLFOX

The development of combination therapy has prolonged the median survival length of patients with advanced colorectal cancer (CRC) to more than 25 months (1). A common treatment strategy consists of combination therapy with a fluoropyrimidine plus oxaliplatin or irinotecan (FOLFOX or FOLFIRI) as first-line treatment, followed by a second-line treatment using the regimen not selected in the first-line treatment. Evidently, 'the use of all three active drugs in advanced CRC produces the longest overall survival (OS)' (2). In addition, the introduction of molecular-targeted drugs, such as anti-vascular endothelial growth factor and anti-epidermal growth factor receptor (EGFR) antibodies, has further improved the prognosis of patients with metastatic CRC (1).

Continuation of bevacizumab, an anti-vascular endothelial growth factor antibody, in the second-line treatment after first-line combination therapy with a conventional cytotoxic doublet and bevacizumab is a widely accepted clinical practice (3). However, the efficacy and safety of the uninterrupted use of an anti-EGFR antibody, such as panitumumab, in the second-line treatment after disease progression during first-line treatment with anti-EGFR antibody remains to be determined. In contrast to bevacizumab, which elicits its anti-tumor effect only in combination with cytotoxic agents, anti-EGFR

antibody alone has clinical benefits in tumor response, OS, and progression-free survival (PFS) (4). Thus, the progression of disease during first-line treatment with cytotoxic doublets and anti-EGFR antibody suggests the development of resistance against both agents to various degrees.

Nevertheless, the potential benefit of uninterrupted (continuous) use of anti-EGFR antibody in second-line therapy cannot be denied, especially when irinotecan-based regimens are saved for second-line therapy. A randomized controlled trial was conducted to compare anti-EGFR monotherapy (cetuximab) and irinotecan plus anti-EGFR combination therapy in 329 patients whose disease had progressed during or within 3 months after irinotecan-based treatment (5). The authors reported that the response rate in patients with combination therapy was significantly higher than that observed during irinotecan monotherapy (22.9 vs. 10.8%, P=0.007). The median time to progression was also significantly longer when combination therapy was administered (4.1 vs. 1.5 months, P<0.001). The result suggests that cetuximab influences the mechanism enhancing the effect of irinotecan (5). Specifically, the authors speculated from preclinical laboratory studies that the inhibition of EGFR by cetuximab may have disabled drug efflux (6), augmented apoptosis induced by irinotecan (7), or weakened DNA-repair activity (8), resulting in the enhancement of the effect derived from irinotecan.

However, the efficacy and safety of the continuous use of panitumumab in first- and second-line treatments for CRC have yet to be determined. We consider that the present study, which addresses this point, will contribute to the further understanding of tumor biology and the improvement of second-line treatment strategies.

Acquired resistance to anti-EGFR antibodies and liquid biopsy

Even in patients with *RAS* wild-type CRC, the clinical efficacy of anti-EGFR antibodies is limited by the development of acquired (secondary) resistance (9). Preclinical models have shown that some alterations in genetic status allow selective tumor cells to grow under anti-EGFR therapy, which raises interest about whether or not these mechanisms are also active in clinical samples. However, analyzing pre- and post-treatment tissue to clarify this question has been hampered due to the invasive nature of the biopsy procedure, which is often unnecessary for individual patients. In addition, identifying the mechanisms of resistance would be quite difficult in view of the intra-tumor heterogeneity, because multiple biopsies within the tumor of interest would be required.

As a breakthrough for this problem, the measurement of circulating tumor DNA (ctDNA), termed 'liquid biopsy', is gaining attention. First, a study revealed that tumor-related ctDNA was found in the majority of patients with metastatic CRC. In addition, the analysis of tumor-related ctDNA can identify a specific mutant genotype, and provide an estimation of total tumor burden (10). Furthermore, Diaz *et al* found that one third of the patients developed multiple different *KRAS* mutations in their sera between 5 and 6 months following treatment, even though their tumors were initially *KRAS* wild-type. This result suggests that mutant *KRAS* genes are released into the circulation with the emergence of resistance to anti-EGFR antibodies (11).

Table I. Endpoints.

Endpoints	Variables
Primary	6-month progression-free survival rate[a]
Secondary	Overall survival
	Progression-free survival[b]
	Time to treatment failure
	Overall response rate
	Disease control rate
	Relative dose intensity
	Safety and tolerability
	Gene mutation status

[a]6-months progression-free survival rate is defined as the proportion of patients who are alive and progression-free survival at 6 months; [b]progression-free survivalis defined as the time from the date of enrollment to the earlier of the date of confirmed progression or death from any cause in the present study.

The measurement of ctDNA enables us to continuously monitor gene mutation status, and may predict the clinical benefit of ongoing anti-EGFR antibody therapy. However, *KRAS* mutations in ctDNA were only identified in a small subset of patients, leaving the mechanism of acquired resistance unexplained. Thus, the present study will investigate the mutation status of other tumor-related genes, including extended *RAS* genes. The results should provide additional information regarding the mechanism of acquired resistance of CRC against anti-EGFR antibodies, and ultimately lead to the establishment of personalized cancer treatment.

Study design and objectives

This study is a multicenter single-arm Phase II clinical trial. The objective is to evaluate the effect of FOLFIRI with panitumumab as the second-line chemotherapy in patients with advanced and/or recurrent CRC, after failure of the first-line treatment with FOLFOX with panitumumab, using the 6-month PFS rate as the primary endpoint. OS, PFS, time to treatment failure, overall response rate, disease control rate, safety, and gene mutation status will be evaluated as secondary endpoints (Table I).

Study population

Patients who meet all of the inclusion criteria and none of the exclusion criteria listed below will be eligible for enrollment in this study.

Inclusion criteria are: a) Histologically-confirmed inoperable colorectal adenocarcinoma excluding vermiform appendix cancer and anal canal cancer; b) Refractory tumor with measurable disease according to the Response Criteria in Solid Tumors (version 1.1) (12) that has progressed, or the appearance of an evaluable new lesion after first-line therapy with FOLFOX plus panitumumab; c) age ≥20 years at the time of informed

Table II. Dose and schedule for FOLFIRI and panitumumab treatment.

Drug	Initial dose	Regimen	Day of administration
Panitumumab	6 mg/kg	i.v. 90-30 min	Day 1
CPT-11[a]	150 mg/m^2 (125 mg/m^2)	i.v. 90 min	Day 1
l-LV	200 mg/m^2	iv. 120 min	Day 1
5-FU-bolus	400 mg/m^2	i.v. Bolus	Day 1
5-FU-infusional	2,400 mg/m^2	i.v. Continuous (46 h)	Days 1-3

[a]CPT-11 will be started at a dose of 125 mg/m^2 in patients identified as homozygous for *UGT1A1*6* or *UGT1A1*28* or simultaneously heterozygous for *UGT1A1*6* and *UGT1A1*28* at the baseline screening. i.v., intravenous.

consent; d) Eastern Cooperative Oncology Group performance status of 0-2; e) written informed consent prior to study-specific screening procedure; f) life expectancy of at least 90 days; g) withdrawal from first-line chemotherapy using FOLFOX plus panitumumab for *RAS* wild-type advanced or metastatic CRC due to intolerable toxicity (but not to panitumumab alone) or progressive disease; h) adequate organ function according to the following laboratory values obtained within 14 days before enrollment (excluding patients who received blood transfusions or hematopoietic growth factors within 14 days before the laboratory test): i) Neutrophil count: ≥1,500/mm^3, ii) platelet count: ≥10.0x10^4/mm^3, iii) hemoglobin: ≥8.0 g/dl, iv) total bilirubin: ≤1.5 mg/dl, v) aspartate aminotransferase, alanine aminotransferase: ≤100 IU/l (≤200 IU/l if liver metastases present), and vi) serum creatinine: ≤1.5 mg/dl; and h) confirmed RAS wild-type tumor at the time of first-line therapy

Exclusion criteria are: a) History of other malignancy with a disease-free interval of <5 years (other than curatively-treated cutaneous basal cell carcinoma, curatively-treated carcinoma *in situ* of the cervix, and gastroenterological cancer confirmed to be cured by endoscopic mucosal resection); b) massive pleural effusion or ascites requiring intervention; c) radiological evidence of brain tumor or brain metastases; d) active infection including hepatitis; e) any of the following concurrent diseases: i) gastrointestinal bleeding or obstruction (including paralytic ileus), ii) symptomatic heart disease (including unstable angina, myocardial infarction, or heart failure), iii) interstitial pneumonia or pulmonary fibrosis, iv) uncontrolled diabetes mellitus, and v) uncontrolled diarrhea (that interferes with daily activities despite adequate therapy); and f) any of the following medical history: i) myocardial infarction: History of one episode within one year before enrollment or two or more lifetime episodes, ii) serious hypersensitivity to any of the study drugs, iii) history of adverse reaction to fluoropyrimidines suggesting dihydropyrimidine dehydrogenase deficiency, iv) previous treatment with irinotecan hydrochloride, v) current treatment with atazanavir sulfate; vi) previous treatment with tegafur, gimeracil, and oteracil potassium within seven days before enrollment, vii) pregnant and lactating females, and males and females unwilling to use contraception, viii) requires continuous treatment with systemic steroid, ix) psychiatric disability that would preclude study compliance, and x) Otherwise determined by the investigator to be unsuitable for participation in the study.

Table III. Dose reduction levels.

Treatment	Starting dose	Level-1	Level-2
Bolus 5-FU (mg/m^2)	400	0	0
Infusional 5-FU (mg/m^2)	2,400	2,000	1,600
Panitumumab (kg/kg)	6	4.8	3.6
CPT-11 (mg/m^2)	150	125	100
If any identified *UGT1A1* polymorphisms as below:	125	100	75
Homozygous for *6			
Homozygous for *28			
Simultaneously heterozygous for *28 and *6			

Treatment plan

Treatment with the FOLFIRI and panitumumab regimen will be continued in 2-week cycles (Table II) until disease progression, unacceptable toxicity and/or patient refusal. Dose reduction will be carried out according to the protocol (Table III).

Measurements

Evaluation of clinical parameters. Evaluation of patient status will be performed according to the evaluation schedule (Table IV). Tumor response will be evaluated according to the Response Criteria in Solid Tumors (version 1.1) (12). Adverse events and adverse reactions will be graded according to the National Cancer Institute-Common Terminology Criteria for Adverse Events (version 4.0). The grade (0-4) with a definition that most closely reflects the finding should be selected.

Sample collection for liquid biopsy. Primary tumor and/or metastatic site tissue samples will be collected in formalin-fixed paraffin-embedded specimens at the time of registration for this clinical trial. Blood samples will be collected at 3 time-points: i) Before second-line treatment; ii) after the first evaluation of chest, abdominal, and pelvic

Table IV. Evaluation schedule.

Examinations	Before enrolment		During the protocol treatment	After discontinuation of protocol treatment
	Within 28 days	Within 14 days		
RAS genotyping	Should be assessed before enrollment			
UGT1A1 genotyping	X			
HCV antibodies, HBs antigen	X		As appropriate	
Sex, age	X			
Medical history, concurrent disease, concomitant drugs	X		X	X1
Imaging assessments: CT, MRI	X	At 6 (±2), 12 (±2), 18 (±2), 24 (±2) weeks and every 8 (±2) weeks, thereafter		X1
Tumor markers: CEA, CA19-9	X			X1
Height, body weight, body temperature		X	As appropriate	As appropriate
ECOG PS		X	X	X2
Subjective and objective findings		X	X	X3
Blood counts, blood chemistry, blood pressure		X	X	X3
PT-INR		X	If clinically indicated	X3
12-lead ECG	If clinically indicated			
Head CT/MRI	If clinically indicated			
SpO2, arterial blood gas, plain chest x-ray	If clinically indicated			
Blood sampling for circulating free DNA	Before start of 1st course		After the 1st CT imaging	After confirm PD

X, performed; X1, response will be assessed every 8 (±2) weeks after discontinuation of protocol treatment in patients without progression; X2, PS will also be evaluated at the start of follow-up treatment; X3, adverse events occurring within 30 days after treatment discontinuation will be followed until recovery.

computed tomography scans (at 6±2 weeks after initiation of the treatment protocol); and iii) after confirmation of acquired resistance to this second-line therapy (between the date of discontinuation of the treatment protocol and initiation of the next-line treatment).

The relationship between the acquired resistance to the first-line treatment containing panitumumab and the genetic mutation detected in the liquid biopsy has yet to be determined in this study. Some of the patients are expected to have acquired mutations at the time of failure during the first-line treatment (sample 1). Some patients could show favorable response to this second-line therapy. However, despite the ongoing tumor response, some of the patients may already have acquired secondary gene mutations at the first tumor evaluation as shown by computed tomography scans (sample 2). If tumor progression is consistently observed following the emergence of gene mutations, the result of the liquid biopsy will become a predictor of tumor response. After acquiring resistance to the second-line chemotherapy (sample 3), the frequency of patients with acquired gene mutations is expected to be higher compared to the initial evaluation (sample 1). At the same time, an alternative mechanism of tumor resistance against panitumumab will be explored.

Evaluation of gene mutations and methylation status. Circulating cell-free DNA will be extracted from 200 μl of plasma using QIAamp Blood Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Amplicon-Based Deep Sequencing will be performed to analyze multiple targeted hotspots in genes that play a key role in the EGFR-MAPK pathway, as well as conventional Sequencing and polymerase Chain Reaction-Reverse Sequence-Specific Oligonucleotide (PCR-rSSO). Furthermore, evaluation of the methylation status of target genes and loci will be performed by using a fluorescence high-sensitive assay (Hi-SA) and bisulfite-modified DNA template as previously described (13).

Statistical considerations

Sample size. The objective of the primary analysis of the study is to confirm a similar additive effect for panitumumab in the second-line therapy for CRC, as reported by Peeters *et al* (14).

In that trial, the median PFS was 4.6 months for FOLFIRI and 6.4 months for panitumumab plus FOLFIRI in the *RAS* wild-type subpopulation. We assumed the threshold and expected 6-month PFS rate as 35 and 50%, respectively. Under the settings of one-sided α=0.10 and power=80%, the required sample size is 53 patients. The target number of cases in this study is 55 patients, considering a dropout rate of 5%.

Rationale for endpoints. PFS is known as a validated surrogate endpoint for metastatic CRC using molecular-targeted agents (15). In a single-arm Phase II trial, the PFS rate at a specified time-point is a simple and frequently used endpoint to summarize the PFS. A 6-month PFS rate, defined as the proportion of patients who are alive and progression-free at 6 months, was set as the primary endpoint in this analysis, because it shows a good correlation with the 1-year OS rate at the individual level (16).

Ethical aspects

The study will be conducted according to the approved protocol and in compliance with the principles of the Declaration of Helsinki, International Conference of Harmonization-Good Clinical Practice, and the 'Ethical Guidelines for Medical and Health Research Involving Human Subjects', and comply with the regulations in Japan. The Institutional Review Board of the Epidemiological and Clinical Research Information Network (17-0601-1) approved the protocol. The protocol and patient information sheet used to conduct the study must be approved by the Institutional Review Board at each study site prior to the start of study participation. After the study has been explained with sufficient time for consideration, if the patient voluntarily agrees to participate in the study, written informed consent will be obtained.

Study organization and funding information

The study is conducted with the Epidemiological and Clinical Research Information Network-related study group and financially supported by Takeda Pharmaceutical Co., Ltd. Although the study will be conducted with drugs manufactured and marketed by Takeda Pharmaceutical Co., Ltd., the company will not play any role or have any authority in the analysis of results, interpretation, report writing, or decisions for data disclosure.

Conclusion

FOLFIRI with panitumumab is recognized as one of the standard second-line therapies for advanced and/or recurrent CRC. Clarifying the benefit and safety of continuous administration of panitumumab with FOLFILI after tumor progression during first-line FOLFOX plus panitumumab treatment will further improve second-line treatment strategies. Evaluation of gene mutation status by liquid biopsy will provide distinctive evidence concerning treatment efficacy and unveil the mechanism underlying resistance development. Ultimately, the information gained from this study is expected to lead to the establishment of a biomarker for tumor response during second-line therapy with panitumumab. The results of the present study will be disseminated at related international congresses and in peer-reviewed journals.

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Availability of data and materials

Not applicable.

Authors' contributions

All authors read and approved the final manuscript. The specific contributions of each author are as follows: HirM, NN, TN, KO, HidM, TK, KY, KM and JS designed the study, produced the study protocol and performed a literature search; KO performed statistical considerations; TN produced the study protocol focusing on biomarkers; NN and JS produced the study protocol focusing on the clinical aspects; and HidM, TK, KY, KM and JS revised the manuscript.

Ethics approval and consent to participate

The study protocol was approved by the Institutional Review Board of the Epidemiological and Clinical Research Information Network (17-0601-1).

Patient consent for publication

Not applicable.

Competing interests

NN has received honoraria for lectures from Takeda Pharmaceutical Company Ltd. KO has received honoraria for lectures from Takeda Pharmaceutical Company Ltd., Bristol-Myers Squibb Company Ltd., Ono Pharmaceutical Co. Ltd., and Chugai Pharmaceutical Co. Ltd. HirM has received honoraria for lectures and research funding from Takeda Pharmaceutical Company Ltd., Chugai Pharmaceutical Co. Ltd., Taiho Pharmaceutical Co. Ltd., Daiichi-Sankyo Pharmaceutical Co., and Yakult Honsha Co. Ltd. TK has received honoraria for lectures from Bayer Yakuhin, Ltd., Japan; Eli Lilly Japan K.K., Ltd.; Yakult Honsha Co., Ltd.; Takeda Pharmaceutical Co., and Chugai Pharmaceutical Co., Ltd. KY has received honoraria for lectures from Taiho Pharmaceutical Co., Ltd., and research funding from Ono Pharmaceutical Co., Ltd., MSD, Eli Lilly and Company, Taiho Pharm. Co., Ltd., Nippon Kayaku Co., Ltd., Covidien, Chugai Pharmaceutical Co., Ltd., Kyowa Hakko Kirin Co., Ltd., Johnson & Johnson K.K., Astellas Pharma Inc., Daiichi Sankyo Co., Ltd., Yakult Honsha Co., Ltd. and Takeda Pharmaceutical Co., Ltd., within

a past year outside the submitted work. KM has received honoraria for lectures from Chugai Pharmaceutical Co., Ltd., Takeda Pharmaceutical Company Ltd., Eli Lilly Japan K.K., Merck Serono Co., Ltd., Taiho Pharmaceutical Co. Ltd., Yakult Honsha Co., Ltd., and research grants from MSD Merck., Daiichi-Sankyo Pharmaceutical Co., Ono Pharmaceutical Co., Ltd., Shironogi & Co., Ltd., Kyowa Hakko Kirin Co. Ltd., Gilead Sciences, Inc. JS has received honoraria for lectures from Nippon Kayaku Co., Ltd., Chugai Pharmaceutical Co., Ltd., Tsumura Co., Ltd, and consulting fee from Takeda Pharmaceutical Co., Ltd., within a past year outside the submitted work. All other authors declare that they have no competing interests.

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