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ORIGINAL PAPER

Multicenter open-label randomized phase II study of second-line panitumumab and irinotecan with or without fluoropyrimidines in patients with KRAS wild-type metastatic colorectal cancer (PACIFIC study)

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Abstract

This phase II clinical trial compared the efficacy and safety of second-line irinotecan and panitumumab treatment (IRI + Pmab) with that of irinotecan, fluoropyrimidines and panitumumab treatment (control) in patients with KRAS wild-type mCRC. The primary endpoint was progression-free survival. In addition, early predictive markers of treatment efficacy were explored. Eighty patients were planned to be recruited. Due to a slow accrual rate, only 48 patients were recruited from 2012 to 2016, of which 23 were allocated to the control group and 25 were allocated to the IRI + Pmab group. The median progression-free survival was 254 days (95% confidence interval, 159–306) for control, and 190 days (95% confidence interval, 159–213) for IRI + Pmab (log-rank test, $P = 0.26$). The response rate without confirmation was 21.7% (5/23) for control and 40.0% (10/25) for IRI + Pmab. Neutropenia, leukopenia, and anorexia were the most common Grade 3/4 adverse events, and several early drop-outs from the treatment protocol were observed in the control group. As for the biomarkers, carcinoembryonic antigen and lactate dehydrogenase (LDH) smoothly declined immediately after the initial dosing in patients with a partial response or stable disease. After starting treatment, LDH-1 and − 2 increased, while LDH-4 and − 5 decreased, irrespective of tumor response. However, exceptions were frequent. In conclusion, this study failed to prove the safety and efficacy of irinotecan and panitumumab treatment due to insufficient patient accrual. Although LDH and its isozymes changed after initiation of treatment, their ability to predict the tumor response may not surpass that of carcinoembryonic antigen levels.
The University Hospital Medical Information Network Clinical Trial Registry: UMIN000007658.

Keywords Panitumumab · Irinotecan · Fluoropyrimidine · KRAS

Introduction

In 2013, 1.6 million new cases of colorectal cancer (CRC) were diagnosed worldwide and more than 0.7 million patients died of disease progression [1]. The number of patients is expected to increase to more than 2.2 million for new cases and 1.1 million for deaths by 2030 [2], which means a considerable number of patients will receive chemotherapy for unresectable/metastatic CRC (mCRC). First-line chemotherapies consist of a combination of fluoropyrimidines and oxaliplatin, or fluoropyrimidines and irinotecan [3]. Second-line treatment is a combination of fluoropyrimidine and the agent which is not chosen in the first-line. The order of the use of oxaliplatin or irinotecan is not considered to affect the prognosis of patients; the use of the three key drugs is the most important tip for the improvement of overall survival (OS) [3]. In addition to the cytotoxic doublets, the recent introduction of anti-vascular endothelial growth factor antibody or anti-epidermal growth factor receptor

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(EGFR) antibody therapy has further improved the prognosis of patients with mCRC [4–6], which continues to force oncologists to seek best-practice treatment for patients with mCRC.

The combination of irinotecan and anti-EGFR antibody (cetuximab or panitumumab) has been used in the second- and third-line treatments after oxaliplatin-based chemotherapy in KRAS wild-type patients. Several trials using this regime demonstrated excellent tumor control with an acceptable rate of adverse events (AEs) [7–9]. However, comparison of the clinical efficacy and safety between treatments composed of anti-EGFR antibody/irinotecan with and without fluoropyrimidines as second-line treatment remains to be conducted. Generally, because the goal of second-line chemotherapy is prolongation of OS rather than cytoreduction for conversion surgery, we expected that the subtraction of fluoropyrimidine from the second-line treatment would lead to an overall benefit by reducing the AEs without a significant impairment in the treatment efficacy.

Besides answering the clinical question raised above, biomarkers of tumor response were also explored in the present study. The use of anti-EGFR antibody therapy is confined to patients with RAS wild-type mCRC due to its lack of benefit in RAS-mutated mCRC [6, 10]. However, RAS is not a sufficient marker to predict tumor response and only a small proportion of patients show a clear benefit from the treatment. To date, to select responders to anti-EGFR therapy, several additional markers, such as sidedness of the primary tumor, polymorphisms of related genes, and mutation status, have been proposed, none of which have proved satisfactory [11–13]. Another approach to this problem is to determine the clinical efficacy of cetuximab immediately after treatment initiation so that responders can be treated with cetuximab while non-responders can leave the treatment with minimum loss. To reach this goal, the relationship between tumor response and the changes in serum levels of carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), lactate dehydrogenase (LDH), and isozymes of LDH were continuously evaluated and analyzed.

Methods

Randomization and ethics

This randomized open-label multicenter phase II trial compared the efficacy and safety of second-line panitumumab and irinotecan with/without fluoropyrimidines in patients with KRAS wild-type mCRC after first-line oxaliplatin-based chemotherapy. From April 2012 to March 2016, patients were assessed for eligibility for this study. Eligible patients were assigned to either panitumumab and irinotecan with fluoropyrimidines or panitumumab and irinotecan without fluoropyrimidines in a 1:1 ratio. The stratified randomization was performed by using four variables: history of bevacizumab treatment, Eastern Cooperative Oncology Group performance status (ECOG-PS), first-line treatment (oral fluoropyrimidine or 5-fluorouracil [5-FU]), and length of first-line treatment (>6 months or ≤ 6 months).

This study was approved by the internal review boards of the non-profit organization Epidemiological & Clinical Research Organization (ECRIN), Aichi Medical University, and the participating institutes. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. All patients provided signed written informed consent before enrollment. This study is registered with the University Hospital Medical Information Network Clinical Trial Registry as UMIN000007658.

Patients

Patients eligible to participate in this study were ≥ 20 years of age, had histologically confirmed KRAS wild-type mCRC with measurable lesions according to the Response Evaluation Criteria in Solid Tumors (RECIST) ver. 1.1, had refractory tumor that progressed after first-line therapy with oxaliplatin-based doublet plus bevacizumab, had an ECOG-PS ≤ 1, and had adequate vital organ function with an estimated life expectancy of at least 8 weeks.

Patients were not eligible if they were pregnant or lactating females, had evidence of a brain tumor or brain metastases, uncontrolled diarrhea, gastrointestinal obstruction or paralysis, infectious disease, severe pulmonary disease, severe systematic co-morbid disease, including diabetes mellitus, heart disease, renal failure and liver failure, cancer osteomyelitis, psychiatric disability, Grade 3 or higher neurological disorder, previous history of irinotecan treatment or anti-EGFR treatment, or history of another malignancy with a disease-free interval of <5 years.

Endpoints

The primary endpoint was progression-free survival (PFS), which was defined as the time from the registration date to the date of disease progression or death from any cause. If disease progression was not observed until the last follow-up, the case was censored. If the second-line treatment was stopped due to refusal or AEs, the event and censor were applied irrespective of the existence of following treatment. The secondary endpoints were response rate (RR), OS (time from registration to death from any cause), disease control rate (DCR), and the profile of AEs. Additionally, serum levels of CEA, CA19-9, LDH, and isozymes of LDH were

continuously monitored to explore their potential as markers of the treatment response.

Treatment plan

When the patient was to receive fluoropyrimidine co-administration (control group), the patient and the clinician could choose FOLFIRI (5-FU + irinotecan) or IRIS (tegafur/gimeracil/oteracil [TS-1] + irinotecan) according to their preference. FOLFIRI + panitumumab treatment comprised of 150 mg/m^2/biweekly of irinotecan, 200 mg/m^2/biweekly of L-leucovorin, 400 mg/m^2/biweekly of bolus 5-FU, 2400 mg/m^2/biweekly of infusional (46 h) 5-FU, and 6 mg/kg/biweekly of panitumumab. IRIS + panitumumab treatment was composed of the same dose of irinotecan and panitumumab, and 80 mg/m^2/day of TS-1 from day 1 to day 7. These treatments were provided every two weeks. If the patients were allocated to the panitumumab and irinotecan group (IRI + Pmab group), the same doses of irinotecan and panitumumab were administered without fluoropyrimidines. The treatment was continued until disease progression defined by RECIST ver. 1.1 or the occurrence of unacceptable AEs.

Examination

Evaluation of the baseline tumor had to be performed within 14 days prior to the registration of the patient in the study. Computed tomography was to be performed every 8 weeks after treatment initiation. In the present study, confirmation of a complete response (CR) or partial response (PR) was not required. The patient responses were not reviewed independently in this clinical trial. The safety evaluation was performed according to the Common Toxicity Criteria for Adverse Events ver. 4.0. The serum levels of CEA, CA19-9, LDH, and isozymes of LDH were evaluated immediately before chemotherapy dosing at each site. Because the primary endpoint of the present study was comparison of PFSs between two groups, a minimal follow-up of 2 years after recruitment of the last patient was planned.

Statistical consideration

A study coded as '20050181' revealed 5.9 months of PFS in patients with KRAS wild-type mCRC treated by second-line FOLFIRI plus panitumumab [14]. In the present study, a PFS of 6 months was predicted. The expected and threshold PFSs were set at 7.5 months and 4.5 months, respectively. Accordingly, a target sample size of 40 (36 eligible patients and 10% ineligible patients) for each group was determined to yield a detection power of 90% with an alpha error of 0.05 for each arm. Proportions and 95% confidence intervals (CI) were calculated to determine RR and DCR. Median OS and PFS were calculated using the Kaplan–Meier method. These statistical analyses were performed using IBM SPSS Statistics 19 (IBM, Armonk, NY). The figures were created using KaleidaGraph Ver. 4.5.2 (Synergy Software, Reading, PA), and edited by Photoshop CS2 (Adobe Systems, San Jose, CA).

Results

Baseline demographics

From April 2012 to May 2016, 48 Japanese patients fulfilling the inclusion criteria were recruited from 15 participating institutes. Twenty-five patients were allocated to receive irinotecan and panitumumab without fluoropyrimidine (IRI + Pmab), while 23 patients received combination therapy with irinotecan, fluoropyrimidine and panitumumab (control; Fig. 1; Table 1). In the control group, 16 patients chose FOLFIRI + panitumumab treatment and 7 patients chose IRIS + panitumumab treatment. The proportion of females was 21.7% in the control group, and 36.0% in the IRI + Pmab group. The factors for randomization (history

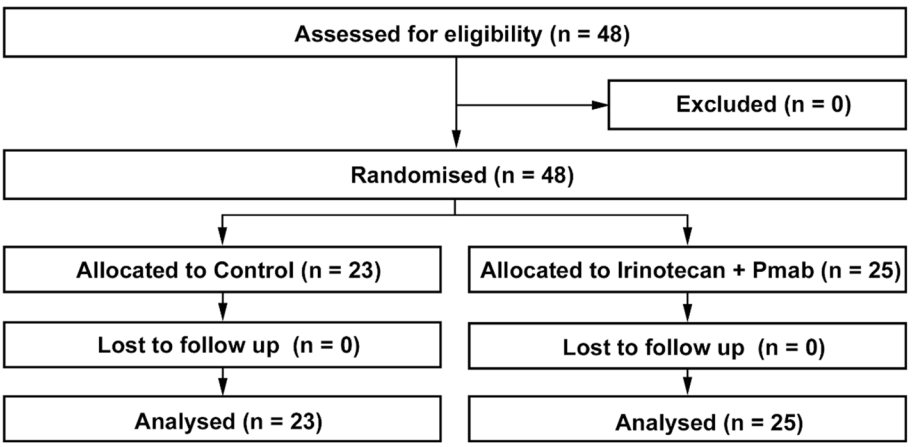

Fig. 1 Flow diagram of the study. Control treatment consisted of panitumumab, irinotecan and fluoropyrimidines. The patients could choose the combination with oral fluoropyrimidine (IRIS), or 5-fluorouracil (FOLFIRI), depending on their preference

Table 1 Characteristics of the patients treated by FOLFIRI/IRIS combined with panitumumab (Control) or irinotecan combined with panitumumab (Irinotecan + Pmab)

	Control ($n=23$)	Irinotecan + Pmab ($n=25$)
No. women	5 (21.7)	9 (36.0)
Age, median (range), years	68 (39–79)	67 (54–81)
Primary tumor site		
Colon	14 (60.9)	18 (72.0)
Rectum	9 (39.1)	7 (28.0)
ECOG performance status		
0	18 (78.3)	18 (72.0)
1	5 (21.7)	7 (28.0)
First-line treatment		
FOLFOX	10 (43.5)	11 (44.0)
XELOX or SOX	13 (56.5)	14 (56.0)
Duration of first-line treatment		
Less than 6 months	9 (39.1)	10 (40.0)
More than 6 months	14 (60.9)	15 (60.0)
Use of bevacizumab in the first-line treatment		
Yes	17 (73.9)	18 (72.0)
Measurable tumor metastasis		
Liver	18 (78.3)	19 (76.0)
Lymph node	8 (34.8)	8 (32.0)
Lung	7 (30.4)	12 (48.0)
Peritoneum	3 (13.0)	2 (8.0)

Data show the number of patients in each group, with percentages in parentheses, unless indicated otherwise

FOLFIRI, 5-fluorouracil + irinotecan; IRIS, tegafur/gimeracil/oteracil + irinotecan; ECOG, Eastern Cooperative Oncology Group; FOLFOX, 5-fluorouracil + oxaliplatin; XELOX, capecitabine + oxaliplatin; SOX, tegafur/gimeracil/oteracil + oxaliplatin

Table 2 Number of treatment cycles and causes of treatment discontinuation

	Control ($n=23$)	Irinotecan + Pmab ($n=25$)
Treatment cycle, *n* (range)	7 (1–21)	9 (2–30)
Reason for treatment discontinuation		
Disease progression	13	17
AEs	2	3
Patient refusal (related to AEs)	3 (1)	3 (2)
Physician decision (related to AEs)	4 (2)	0
Resection of liver metastasis	0	1
Other reason	1[b]	1[a]

Values are numbers of patients, unless indicated otherwise. Control treatment consisted of irinotecan, Pmab, and fluoropyrimidines

Pmab Panitumumab, *AEs* adverse events

[a]Deterioration of performance status

[b]Bone injury not related to treatment protocol

of bevacizumab treatment, ECOG-PS, first-line treatment, and length of the first-line treatment) were similar between groups. Liver was the most common metastatic site, followed by lymph nodes and lungs.

Clinical course and dose intensity

The median number of treatment cycles was 7 (range 1–21) for the control group, and 9 (range 2–30) for the IRI + Pmab group (Table 2). The individual data show that early treatment discontinuation was frequent in the control group (Fig. 2). Of note, the reason for immediate discontinuation after the first cycle was a combination of AEs in two out of these four patients. The major reason for treatment discontinuation was disease progression, accounting for nearly two-thirds of the patients. One patient in the IRI + Pmab

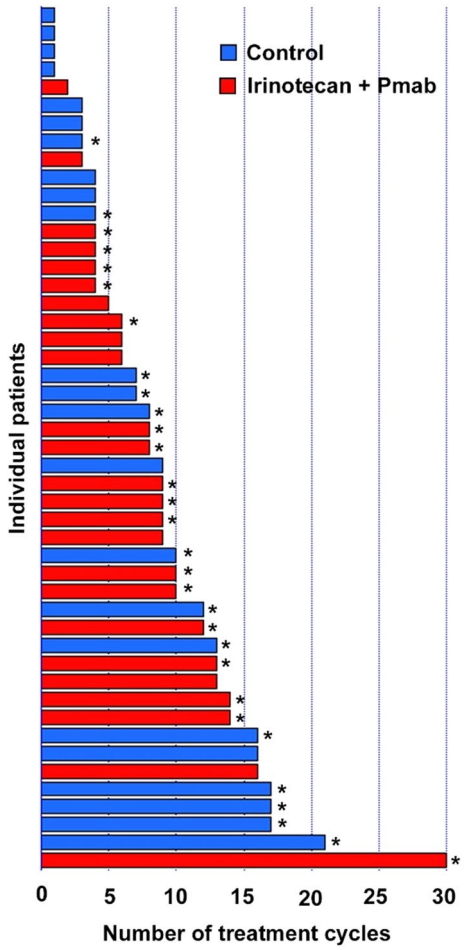

Fig. 2 Individual data of treatment cycle. Control treatment consisted of panitumumab (Pmab), irinotecan and fluoropyrimidines. In the control group, early discontinuation of the treatment protocol was frequently observed. In particular, there were 4 patients who stopped treatment after the first cycle; two of these were related to adverse events. Apart from these patients, the treatment cycles seem equally distributed. The individuals with asterisk (*) had PD

group received curative resection of a hepatic metastasis after 9 cycles of treatment.

After the treatment protocol, seven patients from the IRI + Pmab group did not receive further chemotherapy, while 11 from the control group did not receive further treatment. Among all the patients, eight patients received trifluridine/tipiracil or regorafenib, and in 6 patients oxaliplatin-based treatment was reintroduced. Eight patients continued irinotecan-based chemotherapy after the treatment protocol. Among these eight patients, the addition of fluoropyrimidines to irinotecan with/without bevacizumab was tried in four patients from the IRI + Pmab group.

The relative dose intensity (RDI) of panitumumab and irinotecan for both groups were similar (Table 3). The RDI for TS-1 was as low as 0.59, and we consider that this is related to the drug information saying the initial dosing should be 120 mg/day/body when the body surface area exceeds 1.5 m^2.

Treatment efficacy

The median PFS was 254 days (95% CI 159–306) for the control group and 190 days (95% CI, 159–213) for the IRI + Pmab group, while the median OS was 465 days (95% CI, 225–817) for the control group and 531 days (95% CI, 354–1099) for the IRI + Pmab group, yielding no significant difference between groups for either PFS or OS (Log-rank test, $P = 0.26$ and $P = 0.50$, respectively; Fig. 3). Thirty-four deaths were observed in the study period, mainly due to tumor progression.

There was no CR among the 48 patients (Table 4). Irinotecan and panitumumab treatment yielded a RR of 40.0% and DCR of 87.5%, while control treatment yielded a RR of 21.7% and DCR of 73.9%. The waterfall plot of the change in tumor diameter from baseline shows that the response of the tumor was similar in both groups (Fig. 4). Notably, several patients in the IRI + Pmab group showed substantial tumor shrinkage during the treatment.

Adverse events

Grade 3/4 neutropenia was observed in 6% of the patients in the IRI + Pmab group and 34.8% of patients in the control group (Table 5). Conversely, febrile neutropenia occurred in 16% of patients in the IRI + Pmab group and 4.3% of patients in the control group. Grade 3/4 diarrhea, paronychia, and hypomagnesemia were frequently observed in the IRI + Pmab group, while anorexia, acneiform eruption, and stomatitis were often observed in the control group. No deaths related to AEs were observed.

Changes in CEA, CA19-9, LDH, and LDH isozymes

To find a biomarker predicting tumor response to anti-EGFR antibody therapy, the serum levels of CEA, CA19-9, LDH,

Table 3 Cumulative dose and relative dose intensity (RDI)

	Control ($n = 23$)	Irinotecan + Pmab ($n = 25$)
Panitumumab (Pmab)		
Cumulative dose, mg/kg	42 (5–124)	49 (12–172)
RDI	0.82 (0.50–1)	0.79 (0.49–1)
No. with RDI > 0.8	15	13
Irinotecan		
Cumulative dose, mg/m^2	1052 (143–3144)	1193 (296–4309)
RDI	0.74 (0.43–1)	0.77 (0.37–0.97)
No. with RDI > 0.8	10	10
Bolus 5-FU ($n = 16$)		
Cumulative dose, mg/m^2	2506 (379–8423)	
RDI	0.89 (0.35–1)	
No. with RDI > 0.8	10	
5-FU infusion ($n = 16$)		
Cumulative dose, mg/m^2	15,633 (2289–50,535)	
DI, mg/m^2 per week	0.86 (0.43–1)	
No. with RDI > 0.8	10	
TS-1 ($n = 7$)		
Cumulative dose, mg/m^2	3516 (1132–6964)	
DI, mg/m^2 per week	0.59 (0.45–0.66)	
No. with RDI > 0.8	0	

Values in parentheses are ranges. Control treatment consisted of irinotecan, Pmab, and fluoropyrimidines

5-FU 5-Fluorouracil, *DI* dose intensity, *TS-1* tegafur/gimeracil/oteracil

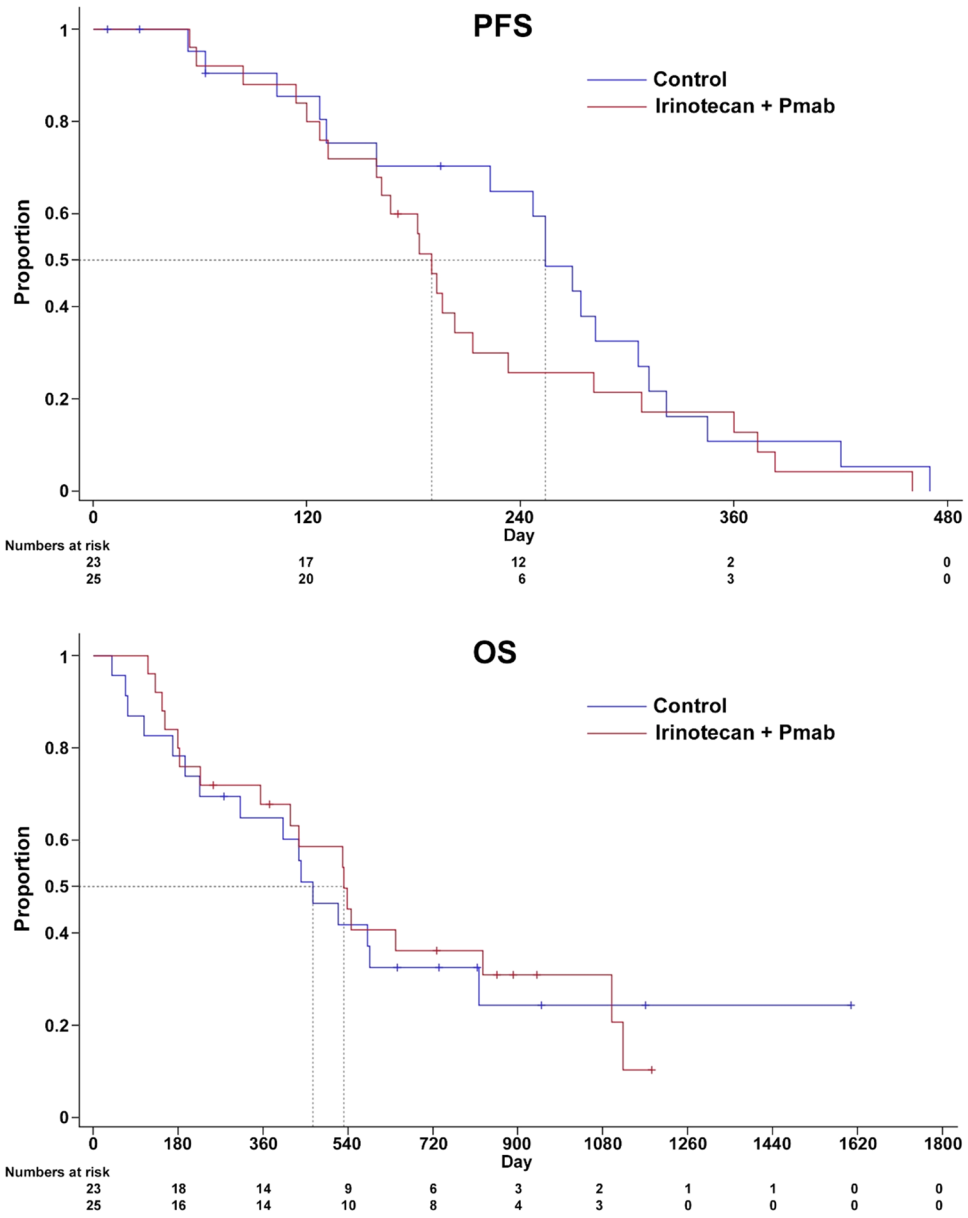

Fig. 3 Progression-free survival (PFS) and overall survival (OS). Kaplan Meier plots for PFS and OS for both control ($n=23$) and irinotecan and panitumumab (Pmab; $n=25$) groups are shown

and isozymes of LDH were obtained immediately before every treatment. The serum levels of CEA and LDH showed a similar trend (Fig. 5). In patients who had PR, the serum level of CEA and LDH decreased before the second cycle of treatment and stayed at a low level. Meanwhile, several patients showed increased levels of CA19-9, compared to baseline, even among the patients with PR. In patients with stable disease (SD), the serum levels of CEA, CA19-9 and LDH showed gradual but staggered changes. In patients with immediate progressive disease (PD), the changes in tumor markers might be characterized as unchanged or small changes either up or down.

To elucidate if the early changes in serum CEA, CA19-9, and LDH predict the tumor response, the change in serum CEA, CA19-9, and LDH from baseline to immediately before the second cycle of treatment was analyzed. The mean ratio (± standard deviation) of the serum level before the second cycle/serum level at baseline was 0.62 ± 0.30 for CEA and 0.71 ± 0.24 for LDH in the patients with PR, while it was 0.90 ± 0.24 for CEA and 1.07 ± 0.52 for LDH in the patients with PD (Table 6).

To analyze the isozymes of serum LDH, missing data immediately before initial treatment or data only obtained at a single point were eliminated. In total the data of 29 patients were analyzed; eight cases of PR, 19 cases of SD, and two cases of PD without PR/SD. The proportion of LDH-1 and LDH-2 increased after the initial treatment, while the proportion of LDH-4 and LDH-5 decreased after the initial treatment; LDH-3 remained the same. However, we could

Table 4 Efficacy of treatment with either FOLFIRI/IRIS combined with panitumumab (Control) or irinotecan combined with panitumumab (Irinotecan + Pmab)

	Control ($n=23$)	Irinotecan + Pmab ($n=25$)
Tumor response		
CR	0	0
PR	5	10
SD	12	11
PD	2	4
NE	4	0
Response rate, %	21.7 (4.9–38.6)	40.0 (20.8–59.2)
Disease control rate, %	73.9 (56.0–91.9)	87.5 (69.6–98.4)
Progression-free survival, days	254 (159–306)	190 (159–213)
Overall survival, days	465 (225–817)	531 (354–1099)

Data are given as the number of patients in each group or as median values with 95% confidence intervals in parentheses

FOLFIRI 5-Fluorouracil + irinotecan, *IRIS* tegafur/gimeracil/oteracil + irinotecan, *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *NE* not evaluated

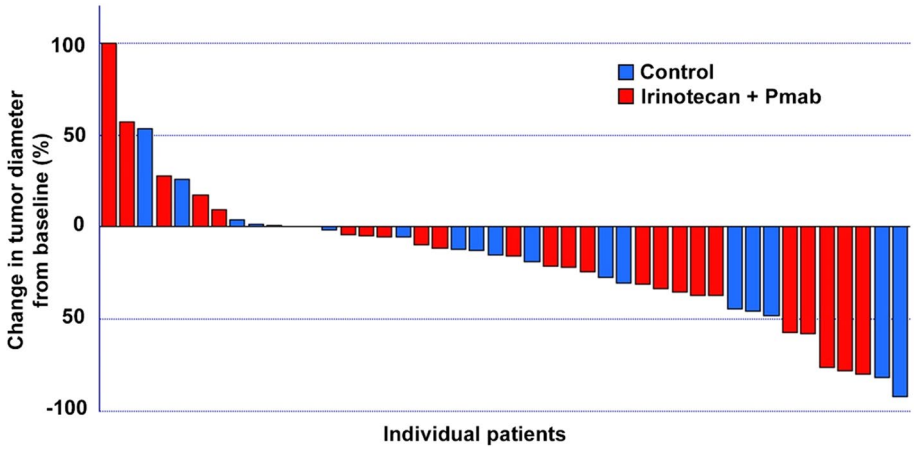

Fig. 4 Change in tumor diameter from baseline. Negative numbers indicate tumor shrinkage during the treatment. A percentage change over 100% was truncated at 100%. Four patients from the control group were not evaluated due to discontinuation of the treatment protocol. Without considering these cases, the distribution of tumor shrinkage appears similar

Table 5 Observed adverse events (AEs) during treatment protocols

	No. Grade 1/2/3/4 AEs		% Grade 3 and 4 AEs	
	Control ($n=23$)	Irinotecan + Pmab ($n=25$)	Control ($n=23$)	Irinotecan + Pmab ($n=25$)
Leukopenia	5/5/3/0	7/8/1/0	13.0	4.0
Neutropenia	5/1/6/2	0/10/2/2	34.8	16.0
Febrile neutropenia	0/0/1/0	0/0/2/1	4.3	12.0
Thrombocytopenia	0/9/1/0	7/0/1/0	4.3	4.0
Fatigue	7/8/1/0	7/5/1/0	4.3	4.0
Nausea	3/5/2/0	7/4/1/0	8.7	4.0
Anorexia	5/8/3/0	7/5/1/0	13.0	4.0
Diarrhea	5/3/2/0	9/5/3/0	8.7	12.0
Acneiform eruption	11/4/2/0	8/14/0/0	8.7	0
Paronychia	1/7/0/0	4/9/4/0	0	16.0
Stomatitis	4/7/2/0	12/4/0/0	8.7	0
Hypomagnesemia	13/2/0/0	8/5/1/2	0	12.0
ALT	11/1/0/0	5/0/0/0	0	0
AST	0/0/0/0	10/2/0/0	0	0
Allergic reaction	18/0/0/0	1/0/0/0	0	0

Control treatment consisted of irinotecan, panitumumab (Pmab), and fluoropyrimidines

ALT Alanine transaminase, *AST* aspartate transaminase

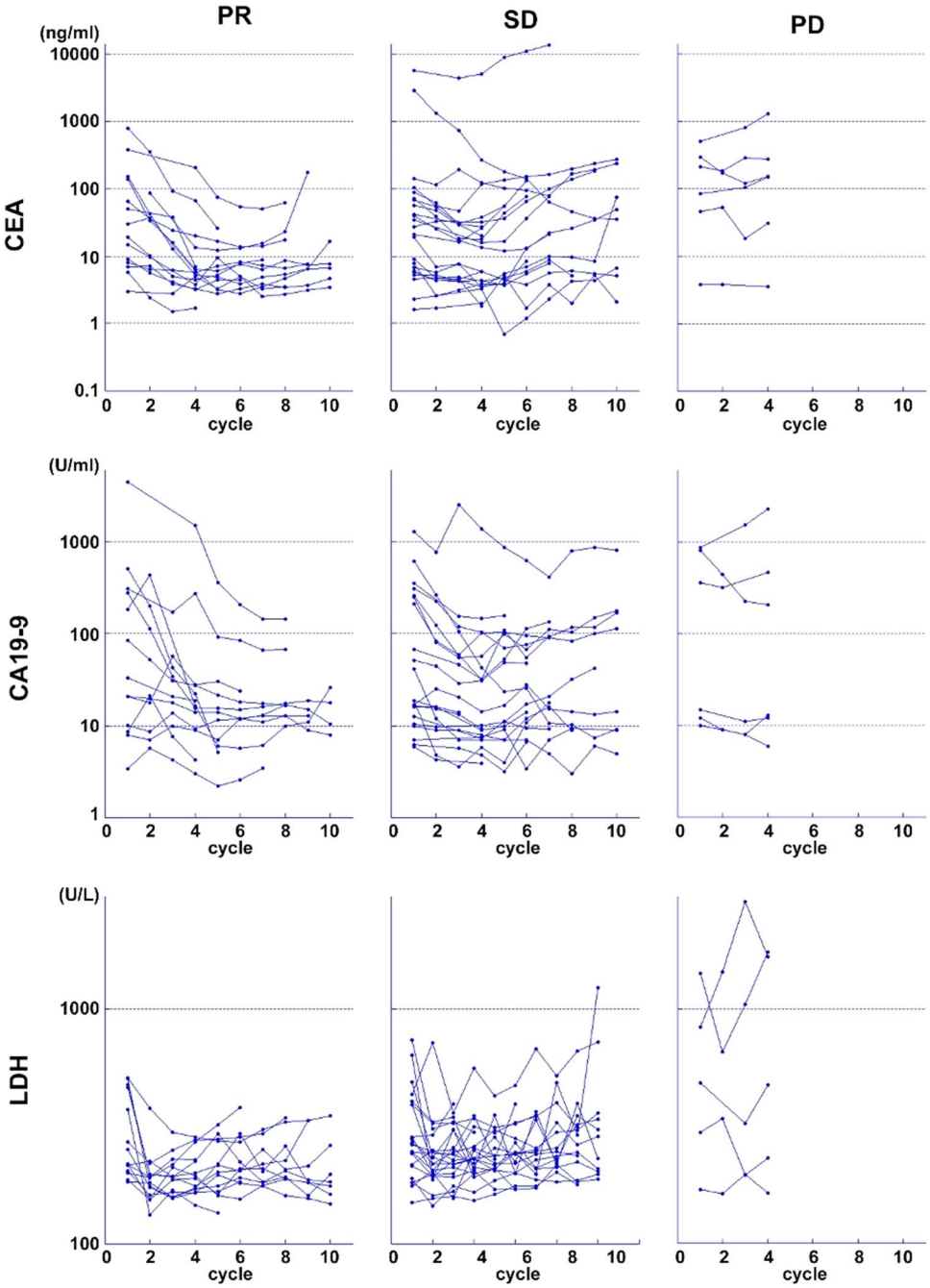

Fig. 5 Changes in carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), and lactate dehydrogenase (LDH). Only values for less than 11 cycles are shown because our focus was on the changes in serum levels early after the start of treatment. Patients with a partial response (PR) show a downtrend and remain low during treatment. In contrast, patients with stable disease (SD) show an up-and-down transition. In patients with progressive disease (PD) without PR/SD, the changes in tumor markers are small or unchanged. This trend was confirmed by the change in serum levels from baseline to immediately before the second cycle

not find any clear differences between the patients with PR, SD, and PD (Fig. 6).

Discussion

This randomized open-label multicenter phase II trial compared the efficacy and safety of second-line panitumumab/irinotecan with and without fluoropyrimidines in patients with KRAS wild-type mCRC after first-line oxaliplatin-based chemotherapy. Although 80 patients were planned to be recruited, the study was terminated after recruiting 48 patients due to a slow accrual rate. The difficulties in recruiting patients in the present setting might have been caused by the concern that removing fluoropyrimidines might have resulted in a reduced efficacy which would be a psychological pressure on patients who have already experienced disease progression or intolerance. Besides, the possibility of conversion to curative surgery after second-line treatment [15] may preclude patients from this type of trial. However, to determine whether these concerns are true or not should be an important role of clinical trials. In the majority of

Table 6 Changes in serum CEA, CA19-9, and LDH levels from baseline to immediately before the second treatment cycle

	Partial response ($n=15$)	Stable disease ($n=23$)	Progressive disease ($n=6$)
CEA			
No. analyzed	11	18	4
Mean ± SD	0.62 ± 0.30	0.73 ± 0.25	0.90 ± 0.24
Median (range)	0.55 (0.25–1.21)	0.74 (0.33–1.02)	0.93 (0.58–1.13)
CA19-9			
No. analyzed	10	16	4
Mean ± SD	1.15 ± 0.76	0.70 ± 0.33	0.77 ± 0.17
Median (range)	0.87 (0.39–2.46)	0.73 (0.26–1.49)	0.82 (0.55–0.90)
LDH			
No. analyzed	14	21	4
Mean ± SD	0.71 ± 0.24	0.86 ± 0.29	1.07 ± 0.52
Median (range)	0.77 (0.35–1.04)	0.83 (0.30–1.66)	1.16 (0.46–1.72)

Data are expressed as (serum level immediately before second cycle)/(baseline serum level). Number of patients analyzed decreased due to missing data immediately before the second cycle

CEA Carcinoembryonic antigen, *CA19-9* carbohydrate antigen 19-9, *LDH* lactate dehydrogenase, *SD* standard deviation

areas, the backbone of first-line chemotherapies is the combination of fluoropyrimidines and oxaliplatin or the combination of fluoropyrimidines and irinotecan [3]. When irinotecan and fluoropyrimidine are administered in the first-line, the use of the fluoropyrimidine in the second-line is 'mandatory' because oxaliplatin alone is not recommended due to the far inferior efficacy of monotherapy compared to combination therapy [16, 17]. In contrast, there is abundant evidence suggesting the clinical efficacy of irinotecan therapy accompanied by anti-EGFR antibody [7–9, 18], which legitimized patient randomization according to our protocol.

Despite the limited data, the present study demonstrates the valuable aspect of irinotecan and panitumumab treatment. First, a smaller number of patients discontinued the treatment protocol promptly after initiation of IRI + Pmab treatment, which suggests this treatment might be more suitable for selected patients. Second, the efficacy of irinotecan and panitumumab treatment could be acceptable when it is used in the second-line. In the control group, the median PFS was 8.5 months, while it was 6.3 months in the IRI + Pmab group. The PFS includes the time after treatment discontinuation to disease progression during third-line treatment if the treatment protocol is discontinued due to the patient's refusal or AEs. Thus, the control group, which had patients with early discontinuation, might have had (although not-statistically significant) a longer PFS than the IRI + Pmab group in the present study. This speculation is strengthened by the OS and RR of the IRI + Pmab and control groups in the present study.

Previously, Sobrero et al. demonstrated that the RR with second-line cetuximab and irinotecan was 16.4% after failure of fluoropyrimidine and oxaliplatin treatment in patients with EGFR-expressing mCRC [7]. Similarly, Carneiro et al. demonstrated a 6% RR during second-line irinotecan and cetuximab treatment among 31 KRAS-unsorted patients [8]. Meanwhile, Kang et al. revealed that the RR among 40 patients with KRAS wild-type mCRC was 45% [9]. In the present study, the RR was 40.0% for the IRI + Pmab group and 21.7% for the control group, which were within the expected range. However, due to the lack of requirement to confirm a CR or PR, and exclusion of KRAS-mutated mCRC, the RR in the present study may have shown a higher RR compared to the previous studies. On the basis that an inter-trial comparison could be harmful, we note that the RR of second-line FOLFIRI and panitumumab treatment in 297 patients with KRAS wild-type mCRC was 36% in a phase III trial [6], suggesting an acceptable RR of irinotecan and panitumumab treatment in the present study.

In the safety analysis, there were no treatment-related deaths in either group. However, different profiles of AEs were observed in the two groups. Grade 3/4 neutropenia and anorexia were frequent in the control group, while paronychia, hypomagnesemia, and febrile neutropenia occurred frequently in the IRI + Pmab group. Analysis of the cumulative dose and RDI for each agent did not reveal a relationship between dose and the different AE profiles. One concern for the control treatment was that two patients in the control group refused to receive further treatment immediately after the initial dosing because of a combination of non-hematological AEs. Otherwise, both treatments seem acceptable in terms of both efficacy and safety.

To explore new biomarkers to predict tumor response at an early phase of treatment, serum levels of LDH and proportions of its isozymes were monitored, in addition to CEA and CA19-9. We observed a dramatic decline in the serum levels of CEA and LDH among patients with PR or

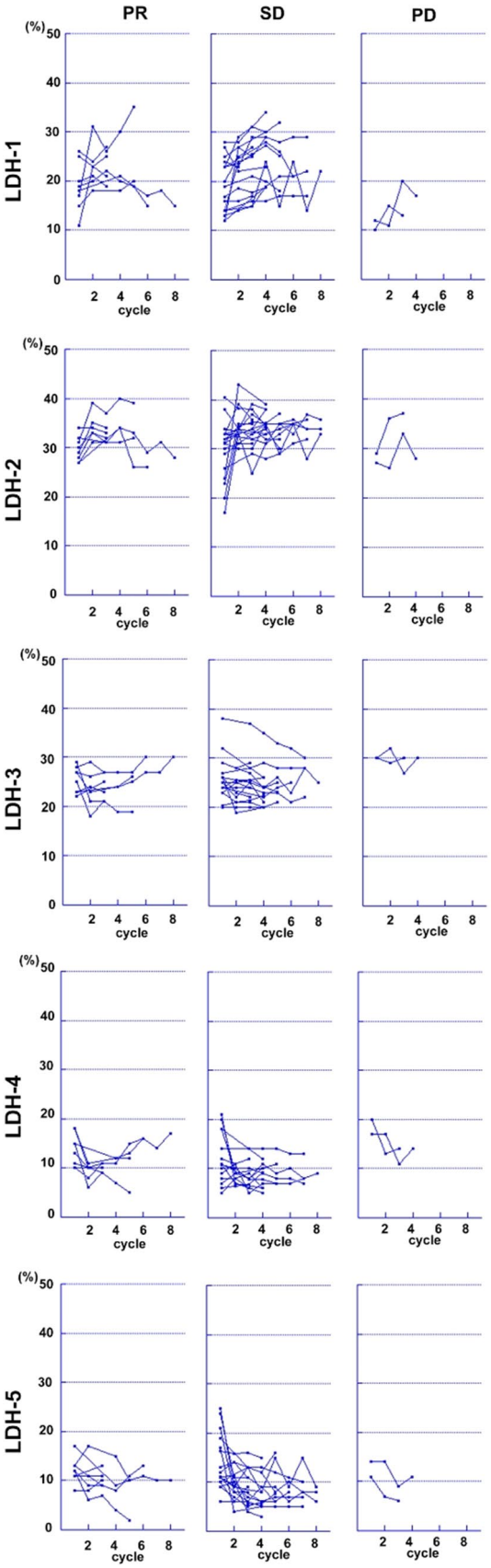

Fig. 6 Changes in lactate dehydrogenase (LDH) isozymes. Changes in the proportions of LDH isozymes are shown for up to 8 treatment cycles. Values after 8 cycles were truncated in the case of 3 patients with stable disease (SD). The proportion of LDH-1 and LDH-2 increased immediately after the initial treatment, while the proportion of LDH-4 and LDH-5 decreased. The level of LDH-3 was stable. PR, partial response; PD, progressive disease

SD. In addition, we noticed that the proportion of LDH-4 and − 5 decreased while that of LDH-1 and − 2 increased after initiation of treatment. Although both normal and cancer cells require glucose as an essential energy source, the metabolism of glucose in tumor cells differs from that observed in normal cells [19]. Compared to normal tissue, tumor cells rely on anaerobic glycolysis in which LDH-5 assumes a crucial role, and LDH-5 is known to be regulated by hypoxia-inducible factors. In contrast, anti-EGFR antibody downregulates hypoxia-inducible factor 1α protein via inhibition of the phosphatidylinositol 3-kinase/Akt and mitogen-activated protein kinase/extracellular signal-regulated kinase pathways [20]. Consequently, the expression of LDH-5 would be reduced in the present study. However, we should also note that elevated or unchanged values of LDH-5 after and during the treatment protocol were not uncommon, revealing the difficulty in using serum levels of LDH or the proportions of LDH isozymes as early predictive markers for tumor response.

Besides the failure to recruit the planned number of patients, another limitation of the present study is the lack of information concerning the sidedness of the primary tumors. As the sidedness of the primary tumor predicts the response to anti-EGFR antibody therapy, the addition of this detail may have led to a more beneficial analysis.

In conclusion, irinotecan and panitumumab treatment yielded a PFS of 6.3 months and a RR, without confirmation, of 40.0% among the present study population. Monitoring of tumor markers, such as LDH and its isozymes, revealed unique conversion patterns although its clinical use could not be established.

Authors' contribution All authors read and approved the final manuscript. The specific contributions of each author are as follows: NN, KI, KH, AM, SI, HT, MI, YY, YM, CT, YM, YH, AT, TN, SM, JS, and HM: conceptualization, data curation; NN, HM, SI, JS, and HM: writing the original draft; NN, HM, SM, JS, and HM: formal analysis, investigation, and methodology; JS and HM: writing - review and editing manuscript.

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Compliance with ethical standards

Conflict of interest NN has received honoraria from Takeda Pharmaceutical Company Ltd. SM has received honoraria (lecture) from Takeda Pharmaceutical Company Ltd., Yakuruto Honsha Ltd., Daiichi-Sankyo Company Ltd. JS has received honoraria (lecture and manuscript fee) from Chugai Pharmaceutical Co. Ltd., Tsumura Co. Ltd., Nihon Kayaku Co. Ltd. and consulting fee from Takeda Pharmaceutical Co. Ltd. outside from this study. Hideyuki Mishima has received speaker honoraria 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. Other authors declare that they have no conflicts of interest.

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