Impact of the individualization of the first-line chemotherapy for advanced colorectal cancer based on collagen gel droplet-embedded drug sensitivity test

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Abstract. Leucovorin (FOL) and fluorouracil (5-FU) plus oxaliplatin (l-OHP; FOLFOX) or FOL and 5-FU plus irinotecan (SN-38; FOLFIRI) are widely used as first-line chemotherapy regimens in the treatment of advanced colorectal cancer (CRC). However, second-line chemotherapy must be abandoned in certain cases due to disease progression, adverse effects or high medical cost. Therefore, the most effective regimen should be selected as first-line chemotherapy. We reported that individualization of first-line treatment (FOLFOX/FOLFIRI/Dual/Poor responder) was possible using the collagen gel droplet-embedded culture drug sensitivity test (CD-DST) and that individualized first-line chemotherapy with CD-DST may improve the prognosis of patients with unresectable CRC. The aim of the present prospective cohort study was to evaluate the individualization of first-line chemotherapy using CD-DST, with a focus on prognosis. Between March 2008 and December 2015, tumor specimens were obtained from 120 patients with CRC who had not received preoperative chemotherapy. CD-DST was performed and the growth inhibition rate (IR) was determined by exposure for 24 h with 5-FU and l-OHP (6.0 and 3.0 μg/ml, respectively) and 5-FU and SN-38 (6.0 and 0.2 μg/ml, respectively). The cumulative distribution of IR values under each condition was evaluated on the basis that the clinical response to FOLFOX and FOLFIRI is equivalent (~50%). The prognosis of dual responder was improved compared with that of poor responders, however this difference was identified to be significant. There was no different prognosis between patients treated with an appropriate first-line regimen and patients treated with an inappropriate first-line regimen in dual responders. However, in poor responders, there were significant differences of prognosis between patients treated with an appropriate first-line regimen and patients treated with an inappropriate first-line regimen (P=0.036). In conclusion, the results from the present study suggest that administration of the recommended first-line regimen using CD-DST for patients with unresectable CRC is important for the improvement of prognosis, particularly in poor responders.

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Introduction

Colorectal cancer (CRC) is one of the most common cancers worldwide (1). Over the last 20 years, and the last decade in particular, the clinical outcome for metastatic CRC patients has improved greatly due to patients undergoing advanced surgical resection of localized metastasis and advanced systemic chemotherapy (2,3). The leucovorin (FOL) and fluorouracil (5-FU) plus oxaliplatin (l-OHP; FOLFOX) or FOL and 5-FU plus irinotecan (SN-38; FOLFIRI) with molecularly-targeted drugs are used as first-line chemotherapy regimens worldwide in the treatment of advanced CRC (4,5). Recently studies have revealed that the median survival time (MST) of advanced CRC with the chemotherapy was >30 months with the integration of multiple cytotoxic agents and molecularly-targeted therapies (6-8). It is common knowledge that the treatment period of the first-line chemotherapy is the longest, and that the response rate of the first-line chemotherapy is the highest (9). However, second-line chemotherapy must be abandoned in certain cases due to disease progression and adverse effects. In addition, high medical costs have been reported to be a significant problem (10-14). Therefore, a more effective regimen should be selected as first-line chemotherapy treatment in a clinical setting.

A previous report demonstrated that individualization of first-line chemotherapy was possible using the collagen gel droplet-embedded culture drug sensitivity test (CD-DST) and individualized first-line chemotherapy using CD-DST may improve the prognosis of patients with unresectable CRC (15,16).

The aim of this prospective cohort study was to evaluate the individualization of first-line chemotherapy using CD-DST, focusing on prognosis.

Materials and methods

Patients. During the period between March 2008 and December 2015, tumor specimens were obtained from 120 patients with CRC. Lymph node metastasis and/or distant metastasis was reported in these patients. No patient was treated with preoperative chemotherapy or chemoradiotherapy. Written informed consent for measurement of individual chemosensitivity was obtained from all patients. Approval for the present study was obtained from the Tobu Chiiki Hospital Institutional Review Board (No. 02.03.29. #1).

Methods. The CD-DST was performed using a Human Cancer Primary Culture System Kit; Primastarä (Kurabo Industries, Ltd., Chuo-ku, Osaka, Japan). Tumor tissue was excised from primary surgical specimens and subjected to the CD-DST. The CD-DST allows for the evaluation of drug sensitivity using isolated 3-dimensionally cultured tumor cells in a small collagen gel droplet, and was used to evaluate the sensitivity of the tumors to 5-FU, which was performed according to a previous description by Kobayashi *et al* (17,18). Each specimen was washed 5 times with 50 ml saline, followed by additional washing 5 times with 50 ml antibiotic fluid containing 1.0 mg/ml piperacillin and 0.5 mg/ml kanamycin. The transport bottle contained 1.0 mg/ml piperacillin, 0.5 mg/ml kanamycin and 2.5 μg/ml amphotericin B. Tissue (1 g) was treated for 2 h at 37˚C with a cocktail containing 1.0% dispersion enzyme EZ™ (Kurabo Industries, Ltd.). Dispersed cell suspensions were inoculated into pre-culture media on collagen-coated flasks (CG-flusk™, Kurabo Industries, Ltd.) overnight. Viable tumor cells were subsequently recovered by 0.05% collagenase treatment. Recovered cells were embedded in 30 μl collagen gel droplets.

The embedded cells were cultivated in culture media containing 5-FU and l-OHP at 6.0 and 3.0 μg/ml (FOLFOX regimen), or 5-FU and SN-38 at 6.0 and 0.2 μg/ml (FOLFIRI regimen), respectively, for 24 h at 37˚C. Following the removal of the anticancer agent-containing media, cells were additionally cultured for 7 days in serum-free culture media (PCM-2™, Kurabo Japan) to prevent the growth of fibroblasts. Viable cells were stained with neutral red solution and counted using the imaging colorimetric quantification method (Primage™, Kurabo Japan). The surviving cell number ratio between the drug-treated and control group, which received no drug treatment, was calculated. A growth rate <0.8 was regarded a successful culture.

The histograms and the cumulative distributions of the growth inhibition rates (IRs) under the two conditions were evaluated based on the evidence that the clinical response rates to FOLFOX and FOLFIRI were ~50% (9,19-21). Therefore, taking the median of the histogram as the cut off value in each regimen, the patients were divided into responder and poor responder.

All patients were divided into 4 cohorts: FOLFOX and FOLFIRI responder (dual responder), FOLFOX responder, FOLFIRI responder and poor responder.

All patients were divided into 3 cohorts: FOLFOX recommended, FOLFIRI recommended and the two regimens recommended.

The patients with the chemotherapy were divided into 2 cohorts: Treated with appropriate first-line regimen and treated with inappropriate first-line regimen.

First-line regimens were selected by the attending physician. Frequencies of chemotherapy and prognosis were prospectively evaluated and compared among the cohorts.

Statistical analysis. Histograms were analyzed with the D'Agostino-Pearson omnibus normality test. Frequencies of chemotherapy were compared between the two cohorts using the t-test. The MST was calculated by the Kaplan-Meier method. The overall survival (OS) curves of the two cohorts were compared by the log-rank test. Data are presented as the means ± standard deviation (SD) and were analyzed using GraphPad Prism (version 5.04; GraphPad Software, Inc., La Jolla, CA, USA). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Patient characteristics are demonstrated in Table I. The median follow-up period was 1124 days. The individual growth IRs under each of the two conditions are presented in Table II. Histograms of the individual growth IRs (%) under the conditions of the FOLFOX regimen and FOLFIRI regimen are presented in Figs. 1 and 2, respectively. The median, mean, SD and standard error (SE) of the mean with the FOLFOX regimen were 59.6, 59.5, 16.8 and 1.54, respectively. The median, mean, SD and SE of the mean with the FOLFIRI regimen were 70.0, 67.7, 16.8 and 1.53, respectively. The histograms passed the normality test (α=0.05; FOLFOX regimen, P=0.52; FOLFIRI regimen, P=0.07).

Four cohorts (dual, FOLFOX, FOLFIRI, and poor responder). The 4 cohorts based on the cumulative distribution of the individual growth IRs between the two conditions is shown in Fig. 3. Individualization of first-line chemotherapy was possible in all 120 patients, with dual responder, FOLFOX responder, FOLFIRI responder and poor responder in 53, 8, 8, and 51 patients, respectively. Thirty-nine of the patients eventually received the chemotherapy in dual responder (n=21), FOLFOX responder (n=3), FOLFIRI responder (n=2) and poor responder (n=13). The MST in dual responder and poor responder was 1128 and 810 days, respectively (P=0.119, Fig. 4).

Three cohorts (FOLFOX, FOLFIRI and the two regimens recommended). Individualization of first-line chemotherapy was possible in all 120 patients, with FOLFOX and FOLFIRI showing higher efficacy in 63 and 51 patients, respectively, and

Table I. Patient characteristics.

Variables	Value
Age, years, mean (range)	66.1 (36-83)
Gender	
Male/female	79/41
Histological type	
Papillary adenocarcinoma	2
Well differentiated adenocarcinoma	22
Moderately differentiated adenocarcinoma	80
Poorly differentiated adenocarcinoma	6
Mucinous adenocarcinoma	9
Squamous cell carcinoma	1
Primary tumor site	
Colon/rectum	76/44

equal efficacy in 6 cases (Fig. 5). Thirty-nine of the patients eventually received the chemotherapy in FOLFOX recommended (n=22), FOLFIRI recommended (n=15), and the two regimens recommended (n=2).

Two cohorts (appropriate and inappropriate first-line chemotherapy). Thirty-nine patients with unresectable CRC were treated with chemotherapy. Patients treated with appropriate first-line regimen and those treated with inappropriate first-line regimen were 28 and 11, respectively. All patients treated with inappropriate first-line regimen received FOLFOX therapy (Fig. 5). The MST in patients treated with the appropriate first-line regimen and those treated with an inappropriate first-line regimen was 960 and 506 days, respectively (P=0.218, Fig. 6). In dual responders, the MST in patients treated with the appropriate first-line regimen (n=17) and those treated with the inappropriate first-line regimen (n=4) was 1044 and 1073 days, respectively (P=0.793, Fig. 7). In the poor responder group, the MST in patients treated with appropriate first-line regimen (n=8) and those treated with inappropriate first-line regimen (n=5) was 810 and 337 days, respectively (P=0.036, Fig. 8). The mean frequency of appropriate and inappropriate regimen in the two cohorts was 22.8±5.17 and 11.0±1.98 courses, respectively (P=0.142, Fig. 9).

Discussion

The present study demonstrated three things. Firstly, the prognosis of a dual responder was improved compared with that of poor responders. Secondly, there was no different prognosis between patients treated with the appropriate first-line regimen and patients treated with an inappropriate first-line regimen in dual responders. Thirdly, in poor responders, there were significant differences in the prognosis between patients treated with an appropriate first-line regimen and patients treated with an inappropriate first-line regimen.

The prognosis of dual responders was improved compared with that of a poor responder. However, there no significant difference was identified between the two cohorts. The reason for this may be that the periods of observation of 4 patients

Table II. Growth inhibition rates (%) of FOLFOX and FOLFIRI.

Patient no.	FOLFOX	FOLFIRI
1	80.1	82.9
2	71.3	79.2
3	81.2	83.4
4	60.0	68.7
5	29.9	66.5
6	69.7	89.6
7	58.7	63.2
8	73.0	85.2
9	63.2	75.9
10	77.9	85.5
11	76.3	85.6
12	53.6	62.6
13	41.9	60.7
14	81.3	80.9
15	42.3	70.2
16	84.8	86.8
17	75.9	83.9
18	59.2	76.4
19	69.9	85.5
20	57.0	49.7
21	79.2	83.0
22	86.1	89.1
23	67.3	74.4
24	81.3	85.2
25	60.4	71.9
26	93.4	98.6
27	62.6	84.9
28	58.5	54.8
29	81.2	84.0
30	66.5	73.3
31	81.3	78.1
32	59.9	74.1
33	53.3	65.0
34	49.3	48.3
35	44.7	49.3
36	68.8	72.1
37	59.7	69.4
38	50.8	59.3
39	51.6	56.5
40	57.9	70.2
41	58.5	63.7
42	62.4	72.5
43	15.2	21.5
44	82.9	84.3
45	66.3	68.1
46	46.7	57.2
47	71.3	70.0
48	32.6	43.2
49	56.4	59.2
50	63.4	64.6
51	59.7	55.3

Table II. Continued.

Patient no.	FOLFOX	FOLFIRI
52	46.2	45.4
53	68.7	77.7
54	49.2	42.7
55	31.5	43.8
56	35.5	41.5
57	69.5	76.6
58	57.0	53.7
59	53.7	55.8
60	72.4	83.4
61	29.4	43.8
62	37.7	65.1
63	68.4	77.2
64	47.9	73.7
65	58.8	69.1
66	50.5	76.5
67	74.3	81.9
68	36.4	46.3
69	81.4	82.4
70	68.0	80.9
71	64.3	78.2
72	69.9	83.7
73	40.7	58.0
74	61.4	55.3
75	60.0	79.5
76	44.8	60.2
77	73.3	93.9
78	66.6	79.8
79	27.8	25.5
80	47.7	66.2
81	51.5	62.6
82	59.4	69.9
83	30.6	26.6
84	68.6	75.6
85	66.5	81.8
86	75.0	73.5
87	56.3	57.1
88	58.0	69.0
89	31.0	47.3
90	84.5	89.5
91	42.1	26.9
92	51.1	54.2
93	86.8	92.0
94	54.8	56.9
95	52.0	70.1
96	49.4	71.7
97	23.7	53.5
98	45.4	63.0
99	47.9	52.6
100	64.6	63.5
101	64.9	78.5
102	53.9	61.4
103	45.4	59.9

Table II. Continued.

Patient no.	FOLFOX	FOLFIRI
104	60.1	79.2
105	36.0	37.1
106	59.1	81.3
107	60.1	69.4
108	34.5	39.9
109	39.8	47.3
110	39.0	44.9
111	38.3	43.3
112	39.9	40.3
113	54.6	66.8
114	79.0	86.8
115	88.1	95.0
116	58.6	52.0
117	88.5	94.1
118	84.9	84.6
119	83.3	83.7
120	99.7	99.9

FOLFOX, leucovorin and fluorouracil plus oxaliplatin; FOLFIRI, leucovorin and fluorouracil plus irinotecan.

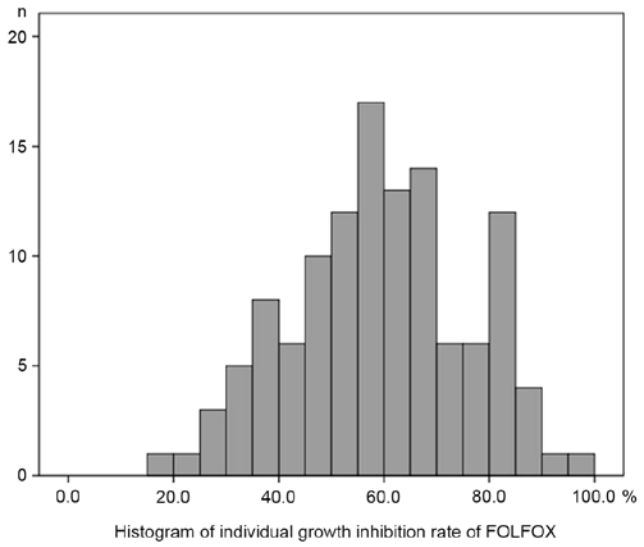

Figure 1. Histogram of individual growth inhibition rate (%) in culture media containing 5-FU and l-OHP at 6.0 and 3.0 μg/ml for 24 h at 37˚C. 5-FU, 5-fluorouracil; l-OHP, l-oxaliplatin; FOLFOX, leucovorin and fluorouracil plus oxaliplatin.

in the dual responder group were <150 days. In the dual responder group, the longest-term survivor (>2700 days) was treated with an inappropriate first-line regimen. However, the patient's growth IRs of FOLFOX and FOLFIRI were 77.9 and 85.5%, respectively. These growth IRs were high level. For certain patients, whose growth IRs of FOLFOX and FOLFIRI were high-level, it was not significant whether FOLFOX or FOLFIRI was administered first. This result may support Grothey's report in dual responders. Several studies have

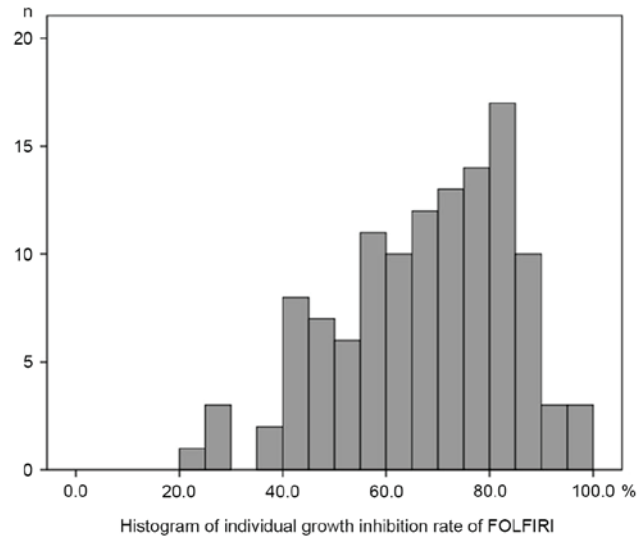

Figure 2. Histogram of individual growth inhibition rate (%) in culture media containing 5-FU and SN-38 at 6.0 and 0.2 μg/ml for 24 h at 37˚C. 5-FU, 5-fluorouracil; SN-38, irinotecan; FOLFIRI, leucovorin and fluorouracil plus irinotecan.

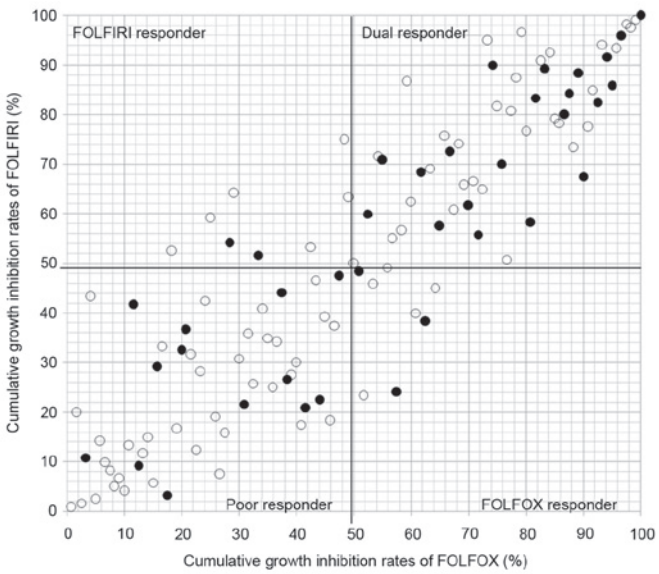

Figure 3. Correlation between cumulative distribution of individual growth inhibition rate between each condition. Solid line indicates cumulative rate 50%. The upper right half area indicates dual responder. The lower right half area indicates FOLFOX responder. The upper left half area indicates FOLFIRI responder. The lower left half area indicates poor responder. Open circle indicates the patients treated without chemotherapy. Closed circle indicates the patients treated with chemotherapy. FOLFOX, leucovorin and fluorouracil plus oxaliplatin; FOLFIRI, leucovorin and fluorouracil plus irinotecan.

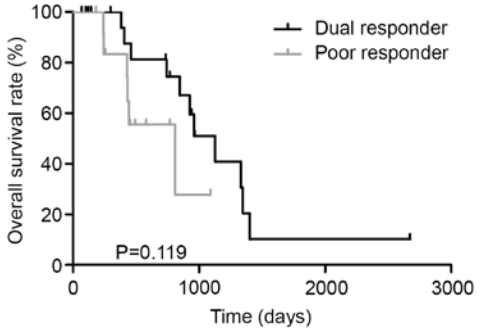

Figure 4. Overall survival rates in dual responder (black line) and poor responder (gray line).

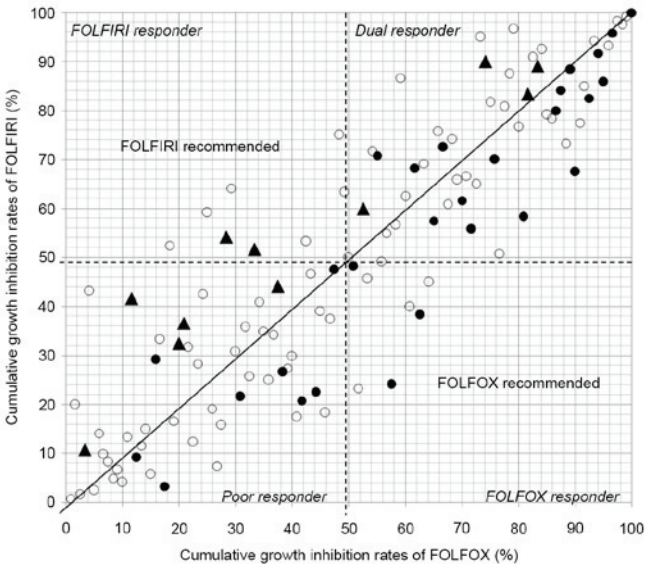

Figure 5. Correlation between cumulative distribution of individual growth inhibition rate between each condition. Solid line indicates equivalence in efficacy for FOLFOX and FOLFIRI. FOLFIRI was superior to FOLFOX in upper left half. FOLFOX was superior to FOLFIRI in lower right half. Open circle indicates the patients treated without chemotherapy. Closed circle indicates the patients treated with appropriate first-line regimen. Closed triangle indicates the patients treated with inappropriate first-line regimen. FOLFOX, leucovorin and fluorouracil plus oxaliplatin; FOLFIRI, leucovorin and fluorouracil plus irinotecan.

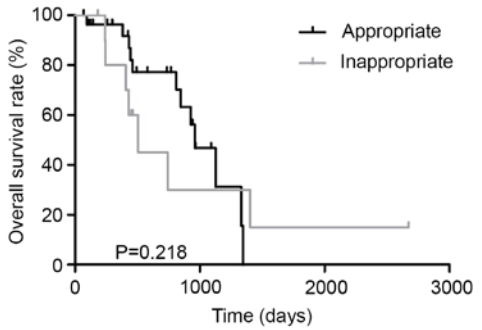

Figure 6. In all patients, overall survival rates in patients treated with appropriate first-line regimen (black line) and patients treated with inappropriate first-line regimen (gray line).

investigated individualization in 5-FU-based chemotherapy based on the 5-FU metabolism-associated enzymatic and genetic characteristics of the individual patient (22-29). In addition, the individualization of 5-FU-based chemotherapy based on the serum 5-FU concentration has been reported lately (30-34). However, individualization in 5-FU-based chemotherapy remains to be implemented in a clinical setting. Therefore, CD-DST may be useful to detect poor responder in 5-FU-based chemotherapy. On the other hand, the individualized chemotherapy with molecularly-targeted anticancer agents may be implemented based on the

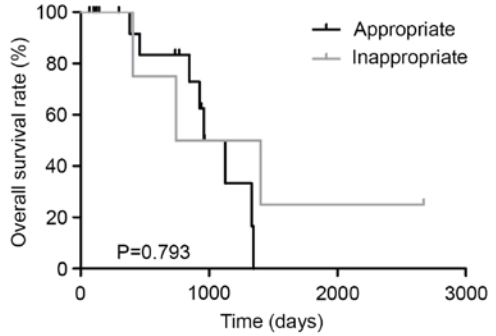

Figure 7. In dual responders, overall survival rates in patients treated with appropriate first-line regimen (black line) and patients treated with inappropriate first-line regimen (gray line).

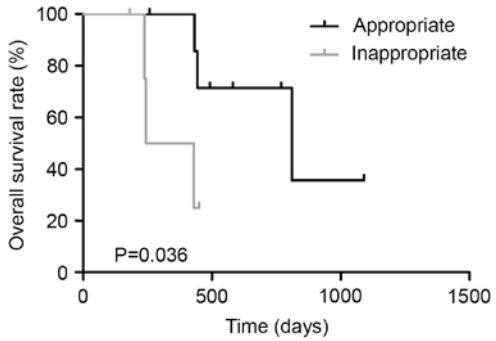

Figure 8. In poor responders, overall survival rates in patients treated with appropriate first-line regimen (black line) and patients treated with inappropriate first-line regimen (gray line).

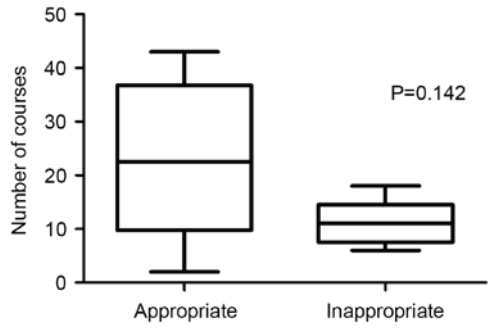

Figure 9. Frequency of chemotherapy of the patients treated with appropriate first-line regimen and the patients treated with inappropriate first-line regimen.

genetic characteristics of the individual patient. Recently, the importance of the biomarker for molecularly-targeted anticancer agents has become increasingly evident in a clinical setting (35-37). In poor responders, the biomarker for molecularly-targeted anticancer agents is required in order to improve the prognosis. Therefore, advanced studies into biomarkers are important.

There was no different prognosis between patients treated with the appropriate first-line regimen and patients treated with the inappropriate first-line regimen in dual responders. Grothey *et al* (38) reported that while it was not significant whether FOLFOX or FOLFIRI was administered first, it was crucial that full administration of the targeted dosages of all 3 drugs (5-FU, oxaliplatin and irinotecan) was achieved (38). However, second-line chemotherapy must be abandoned in certain patients due to disease progression, adverse effects or high medical cost in a clinical setting. The cost of molecularly-targeted anticancer agents in particular is expensive. Therefore, the number of reports on cost effectiveness analysis and cost utility analysis are rapidly increasing (10-14). In randomized controlled trials (RCTs), the rate of second-line chemotherapy enforcement in PRIME study, OPUS study, NO16966 study and FIRE-3 study was 62, <50, 53 and 69.9%, respectively (39-42). Even in those recent RCTs with strict eligible standard, second-line chemotherapy could not be carried out in >30% of the patients. First-line chemotherapy is usually administered over a long period of time (9). In addition, the response rate of the first-line chemotherapy is typically higher compared with second-line chemotherapy (43,44). Therefore, selection of a more effective regimen as the first-line chemotherapy using CD-DST is extremely important even for dual responder patients in a clinical setting. The present study has already reported the following: When the clinical response rates of FOLFOX and FOLFIRI were 50%, responders were identified using the median based on the histograms of the individual growth IRs. The efficacies of FOLFOX and FOLFIRI were not exactly equivalent in all the individuals. By using CD-DST, it was possible to individualize the first line chemotherapy and may also improve the prognosis of patients with unresectable CRC.

In poor responders, there were significant differences of prognosis between patients treated with appropriate first-line regimen and patients treated with an inappropriate first-line regimen. Moreover, more chemotherapy in patients treated with the appropriate first-line regimen was performed. It is crucial to administer the appropriate first-line regimen. Administration of a more effective first-line regimen leads to prolonging the period of first-line chemotherapy and increases the total number of chemotherapy cycles. This indicates the importance for the detection of poor responders and the selection of first-line chemotherapy using CD-DST.

There were certain limitations to the present cohort study. Firstly, the sample size was small; a larger sample size would have improved the quality of the data. Secondly, in the present study, the periods of observation of 4 patients in dual responder were shorter (<150 days) compared with other patients. The short periods of observation may influence statistical analysis. Moreover, the present study was not randomized. The individualization of first-line chemotherapy using CD-DST requires additional prospective randomized studies.

In conclusion, the results from the present study suggest that the administration of the recommended first-line regimen using CD-DST for patients with unresectable CRC is important for improvement in prognosis. It is important to administrate appropriate first-line regimen, particularly in poor responders.

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