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Clinical Predictors of Cetuximab for First-Line Therapy of Metastatic Colorectal Cancer: A Single Institutional Retrospective Study

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Abstract

Predictive factors of cetuximab efficacy for metastatic colorectal cancer (mCRC) have not been sufficiently revealed. The present study aimed to explore new predictors. A total of 30 patients with KRAS exon 2 wild-type unresectable mCRC, who had been treated with cetuximab-based regimen as first-line therapy, were retrospectively analyzed. We assessed whether gender, age, primary tumor site, RAS genotype, the Eastern Cooperative Oncology Group Performance Status (ECOG PS), metastatic status, histological grade, carcinoembryonic antigen (CEA), treatment regimen, and oxaliplatin-based adjuvant chemotherapy at baseline were associated with cetuximab efficacy. Progression-free survival (PFS) and objective response rate (ORR) were evaluated and statistically analyzed. Analysis of PFS revealed that left-sided tumor and good PS had relevance to good results. PFS among patients with left-sided CRC was longer than that among those with right-sided CRC (median, 10.6 and 3.5 months, respectively). Patients with a PS of 0 - 1 experienced significantly longer PFS than those with a PS of 2 - 3 (median, 8.6 versus 1.3 months, respectively). In analysis of ORR, high histological grade and serum CEA level showed interaction with good effect. Patients with histological grade I/II cancer experienced better ORR than those with histological grade III/IV cancer (76% versus 20%, respectively). ORR among patients with serum CEA level higher than 5.0 ng/ml was significantly higher than that among those with lower serum levels (88% versus 38%, respectively). ECOG PS, tumor location, histological grade, and serum CEA level at baseline might be useful predictors of cetuximab efficacy in the first-line treatment of mCRC.

Keywords

Cetuximab, Colorectal Cancer, Clinical Predictor

1. Introduction

Improvement of treatment outcomes to colorectal cancer (CRC) is a matter of public health concern in developed countries. In Japan, CRC is the fourth most common cancer in men (115.9 per 10,000 population) and the second most common in women (80.5 per 100,000 population), and CRC mortality is the third highest in men (42.9 per 100,000 population) and the highest in women (34.6 per 100,000 population) among the mortality rates for various cancers.

Cetuximab is an agent against epidermal growth factor receptor (EGFR). The expression of EGFR is detected with a high probability in CRC, and associated with poor prognosis and survival [1] [2] [3]. Cetuximab, a chimeric IgG1 monoclonal antibody against EGFR, binds to EGFR with high affinity and blocks ligand-induced activation of EGFR. In Japan, this agent was recently approved as an agent against EGFR-positive metastatic or recurrent CRC [4]. More outcome data of cetuximab for Japanese patients in clinical practice is desired.

Some EGFR downstream signal pathways have been revealed to be biomarkers of cetuximab efficacy. KRAS exon 2 mutations are the most common biomarker for predicting the efficacy of anti-EGFR antibodies in advanced CRC [5]. KRAS (exon 3, 4), NRAS (exon 2, 3, 4) and BRAF mutations are also predictive biomarkers [6] [7]. Also, recently, amphiregulin and epiregulin, which belong to the epidermal growth factor (EGF) family, were reported as predictive biomarkers of cetuximab [8] [9]. However, it is difficult to utilize these factors as biomarkers of efficacy in clinical use. The primary tumor site (left- or right-sided) has been demonstrated as a useful biomarker for predicting efficacy of cetuximab treatment in several studies [10] [11]. This is a very clinically useful predictor, and the National Comprehensive Cancer Network (NCCN) Guidelines for Colon Cancer V. 1. 2017 state that panitumumab and cetuximab combination therapy is only recommended for left-sided tumors. However, other convenient clinical predictors have not been sufficiently revealed.

In the present study, we assessed clinical efficacy and adverse effects of cetuximab-based regimen for advanced mCRC. In addition, we explored possible new clinically convenient predictors of this regimen.

2. Patients and Methods

2.1. Patients and Treatments

All 30 patients with KRAS exon 2 wild-type unresectable metastatic CRC (mCRC) who had been treated with cetuximab-based regimen as first-line therapy at Okayama Rosai Hospital between August 2011 and December 2015 were retrospectively analyzed. Patients were treated with cetuximab and either FOLFOX (oxaliplatin, folinic acid, and 5-FU), FOLFIRI (5-FU, folinic acid, and irinotecan), or SOX (S-1 and oxaliplatin). Cetuximab was given at an initial dose of 400 mg per square meter of body-surface area, followed by a weekly maintenance infusion of 250 mg per square meter. All patients had measurable lesions according to Response Evaluation Criteria in Solid Tumors (RECIST; ver-

sion 1.1) [12]. This study was approved by the institutional review board of Okayama Rosai Hospital. Written informed consent was obtained from all patients.

2.2. KRAS Mutation Analysis

Formalin-fixed, paraffin-embedded (FFPE) samples of tumor tissue from archival specimens were collected at the time of diagnosis. DNA samples were obtained from the FFPE samples, and genomic DNA was extracted. Mutations in KRAS were detected using the multiplex PCR-Luminex method-based MEBGEN Mutation Kit (Medical & Biological Laboratories, Nagoya, Japan).

2.3. Assessment

Eastern Cooperative Oncology Group Performance Status (ECOG PS) was used to evaluate each patient's performance status at baseline [13]. The histological grade of CRC was assessed based on the percentage of glandular differentiation in the tumor according to the World Health Organization (WHO) criteria [14]. Radiologic assessments of tumors were performed by investigators every about 8 weeks, and Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 was used to assess tumor responses. Various toxicities (rash, anorexia, malaise, fatigue, nervous system disorders, nausea and vomiting, stomatitis, constipation, diarrhea, and alopecia) were evaluated each time the patients visited the hospital, and graded according to the Common Terminology Criteria for Adverse Events (CTCAE), version 4.0.

2.4. Statistical Analyses

Differences in objective response rate (ORR) between groups were examined using the Fisher's exact test for categorical variables. Progression-free survival (PFS) and overall survival (OS) curves were generated using the Kaplan-Meier method. To uncover clinical predictive factors, the log-rank test was used. Values of $P < 0.05$ were considered to be significant. All statistical analyses were performed with EZR (Saitama Medical Centre, Jichi Medical University), which is a graphical user interface for R (The R Foundation for Statistical Computing, version 3.2.2) [15].

3. Results

3.1. Patient Characteristics

A total of 30 patients with KRAS exon 2 wild-type unresectable mCRC who were treated with cetuximab-based regimen as first-line therapy were included in the study. The sample population comprised 22 males and 8 females, with median age 67 years (range, 32 - 85 years). Among the 30 patients, 23 had left-, and 7 had right-sided CRC; 26 had ECOG PS of 0 to 1, and 4 had PS of 2 to 3; 23 had all-RAS wild-type; and 5 had low (III/IV) histological grade cancer. Carcinoembryonic antigen (CEA) levels at baseline of 17 patients were more than 5.0

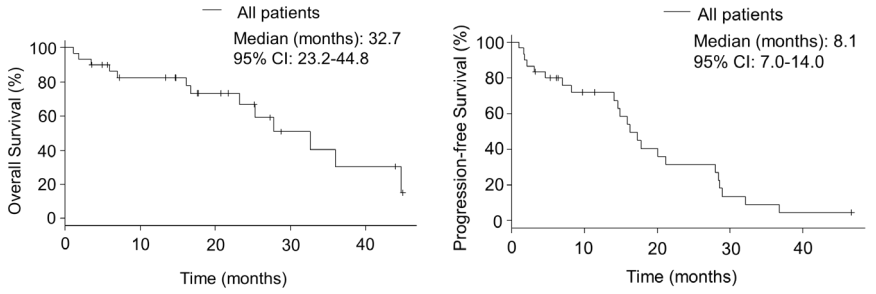
ng/ml. Patients received cetuximab-based regimens including FOLFOX (20 patients), SOX (4 patients), and FOLFIRI (6 patients); 7 patients had experienced oxaliplatin-based adjuvant chemotherapy. The patient background characteristics are listed in Table 1.

3.2. Treatment Outcomes

Clinical outcomes are shown in Table 2. For all patients, the ORR was 67%, and the disease control rate (DCR) was 83%. The median OS was 32.7 months (95% CI, 23.2 - 44.8 months; Figure 1(a)) and the median PFS was 8.1 months (95% CI, 7.0 - 14.0 months; Figure 1(b)). Only 1 patient, who underwent treatment with mFOLFOX6 plus cetuximab regimen, had complete response. At the time of analysis, 27 patients (90%) had already finished treatment, and the median treatment period was 7.3 months (range, 1 - 18 months).

Table 1. Patient characteristics.

Parameter		
Gender, n (%)	Male	22 (73)
	Female	8 (27)
Age (years)	Median (range)	67 (32 - 85)
Primary tumor site, n (%)	Right-sided	7 (23)
	Left-sided	23 (77)
KRAS wild type, n (%)	Yes	30 (100)
	No	0 (0)
Other RAS genotype, n (%)	Wild-type	23 (77)
	Mutant-type	7 (23)
ECOG PS at baseline, n (%)	0 - 1	26 (87)
	2 - 3	4 (13)
Organs with metastases, n (%)	0 - 2	25 (83)
	3	5 (17)
Lymph node metastasis at baseline, n (%)	Positive	15 (50)
	Negative	15 (50)
Peritoneal metastasis at baseline, n (%)	Positive	6 (20)
	Negative	24 (80)
Histological grade, n (%)	1 - 2	25 (83)
	3 - 4	5 (17)
CEA at baseline, n (%)	<5.0 ng/ml	13 (43)
	≥5.0 ng/ml	17 (57)
Treatment regimen, n (%)	FOLFOX	20 (67)
	SOX	4 (13)
	FOLFILI	6 (20)
Oxaliplatin-based adjuvant chemotherapy, n (%)	Yes	7 (23)
	No	23 (77)



(a) (b)

Figure 1. Overall survival and progression-free survival. Kaplan-Meier curves for (a) overall survival among all 30 patients, (b) progression-free survival among all 30 patients.

Table 2. Treatment characteristics.

Parameter		
Treatment response, n (%)	Complete response	1 (3)
	Partial response	19 (63)
	Stable disease	5 (17)
	Progression disease	5 (17)
Progression-free survival (months)	Median	8.1
	95% CI	7.0 - 14.0
Overall survival (months)	Median	32.7
	95% CI	23.2 - 44.8

3.3. Clinical Predictors of Cetuximab-Based Regimen

ORR, PFS, and OS were compared respectively with various factors (Table 3). Factors with a differential outcome of ORR or PFS may reflect predictive markers for cetuximab efficacy.

When factors were analyzed for ORR, high histological grade and serum CEA level showed significant interaction with good clinical effect. Patients with histological grade I/II cancer experienced better ORR than those with histological grade III/IV cancer (76% versus 20%, respectively; $P = 0.031$). ORR among patients with serum CEA level higher than 5.0 ng/ml was significantly higher than that among those with lower than 5.0 ng/ml (88% versus 38%, respectively; $P = 0.0069$).

In analysis of PFS, left-sided tumor and good PS had significant relevance to good results. Median PFS in left-sided CRC patients was 10.6 months, significantly longer than that in right-sided CRC patients (3.5 months; log-rank test, $P = 0.00031$). Patients with a PS of 0 - 1 experienced significantly longer PFS than those with a PS of 2 - 3 (median, 8.6 months versus 1.3 months, respectively; log-rank test, $P = 0.0030$).

3.4. Adverse Effects

Adverse events were evaluable in 28 of 30 patients, and are detailed in Table 4. No adverse events of grade 4 were observed.

Table 3. Association between clinical factors and treatment outcome.

Parameter	n	Treatment response		Progression-free survival		Overall survival	
		ORR (%)	*P* value (Fisher test)	Median (months)	*P* value (log-rank test)	Median (months)	*P* value (log-rank test)
Gender							
Male	22	73	0.38	8.6	0.26	36.0	0.33
Female	8	50		7.0		25.3	
Age (years)							
<75	23	74	0.18	8.6	0.85	27.8	0.47
≥75	7	43		7.3		32.7	
Primary tumor site							
Right-sided	7	43	0.18	3.5	0.00031	16.7	0.025
Left-sided	23	74		10.6		36.0	
Other RAS genotype							
Wild-type	23	70	0.66	10.0	0.089	36.0	0.26
Mutant-type	7	57		7.9		23.2	
ECOG PS at baseline							
0 - 1	26	73	0.095	8.6	0.0030	32.7	0.000013
2 - 3	4	25		1.3		4.4	
Lymph node metastasis at baseline							
Positive	15	73	0.70	8.1	0.40	32.7	0.70
Negative	15	60		8.9		25.3	
Peritoneal metastasis at baseline							
Positive	6	67	1	8.6	1.0	27.8	0.21
Negative	24	67		8.1		32.7	
Histological grade at baseline							
1 - 2	25	76	0.031	8.6	0.13	36.0	0.022
3 - 4	5	20		1.5		23.2	
CEA at baseline							
<5.0 ng/ml	13	38	0.0069	4.1	0.40	23.2	0.0043
≥5.0 ng/ml	17	88		10.0		44.8	
Oxaliplatin-including treatment regimen							
Yes	24	71	0.37	8.1	0.74	36.0	0.069
No	6	50		8.9		23.2	
Oxaliplatin-based adjuvant chemotherapy							
Yes	7	57	0.66	8.1	0.44	32.7	0.79
No	23	70		8.9		27.8	

Table 4. Adverse events.

Adverse Events	Any Grades	>Grade 3
	No. of patients (%)	
Rash	26 (93)	3 (11)
Anorexia	25 (89)	0 (0)
Malaise	25 (89)	0 (0)
Fatigue	23 (82)	0 (0)
Nervous system disorders	23 (82)	0 (0)
Nausea and vomiting	22 (79)	0 (0)
Stomatitis	19 (68)	0 (0)
Constipation	17 (61)	0 (0)
Diarrhea	12 (43)	2 (7)
Alopecia	12 (43)	0 (0)

All patients had experienced an adverse event. The most frequent adverse event was rash, observed among 26 of 28 patients. Grade 3 adverse events observed in the present study were rash (3 patients) and diarrhea (2 patients).

4. Discussion

Personalized treatment of patients with mCRC is becoming routine in clinical practice. Cetuximab-based therapy is among the mCRC treatments, and has been performed in selected patients. Formerly, cetuximab was used for unselected patients with EGFR-positive mCRC based on trial NCIC CTG CO.17 and BOND trial results [4] [16]. Subsequently, significant interactions between KRAS status and cetuximab treatment effect were noted in the CRYSTAL trial, and similar results were obtained from past trials [5] [6]. Further subgroup analysis in CRYSTAL validated all-RAS mutations as negative predictors for cetuximab therapy [7]. Also, recent studies have found that location of CRC (right-sided versus left-sided) is a useful predictor of cetuximab treatment outcome. In trial CALGB/SWOG80405, impact of primary tumor site on OS and PFS in patients with KRAS wild-type mCRC was analyzed [17]. PFS of patients with left-sided primary tumor was significantly longer than those with right-sided primary tumor (12.0 months versus 7.7 months, respectively). Several other studies had reported similar outcomes, and the National Comprehensive Cancer Network (NCCN) Guidelines for Colon Cancer V. 1. 2017 state that panitumumab and cetuximab combination therapies are only recommended for left-sided tumors.

In the present study, we explored predictors using ORR and PFS as indicators of efficacy. In total, 30 patients with KRAS exon 2 wild-type unresectable mCRC received cetuximab-based regimen as first-line therapy. As a result, left-sided CRC, good PS, well/moderately differentiated type, and high serum level of CEA showed relations to good clinical response. Patients with left-sided CRC had sig-

nificantly better PFS than those with right-sided CRC (10.6 months versus 3.5 months, respectively; $P = 0.00031$). These results are similar to those of studies so far reported. Also, patients with a PS of 0 - 1 showed significantly longer PFS than those with a PS of 2-3. Jonker, D. J., *et al.* reported that relative benefits of cetuximab in terms of PFS were seen in subgroups defined on the basis of ECOG PS at baseline in trial NCIC CTG CO.17 [16]. Our study indicated that good PS was related to better clinical outcome as well. Our results showed that patients with histological grade I/II cancer experienced better ORR than those with histological grade III/IV cancer. When patients were limited to all RAS wild-type, ORR among 19 patients with histological grade I/II cancer was higher than in the other 4 patients (79.0% versus 25.0%, respectively). In CRC, some studies showed an association between EGFR expression and tumor differentiation grade [18] [19] [20]. It is suggested that moderately/well differentiated CRC cells express EGFR activity to a greater extent than poorly differentiated cells. This supports our result, that patients with moderately/well differentiated cancer showed better treatment response than those with poorly differentiated cancer on treatment with cetuximab-based regimen. Poor histological grade is considered among the adverse histopathological factors associated with unfavorable clinical course of CRC. In cetuximab treatment, poor histological grade may be not only an unfavorable prognostic factor, but also an adverse clinical predictor. It was also observed that serum CEA level influenced efficacy of cetuximab-based regimen in this study. ORR among patients with serum CEA level higher than 5.0 ng/ml was significantly better than that among those with lower serum levels. When patients were limited to all RAS wild-type, ORR among 13 patients with serum CEA level higher than 5.0 ng/ml was significantly higher than that among 10 patients with lower serum levels (92.3% versus 40.0%, respectively; Fisher test, $P = 0.019$). Patients with high serum levels of CEA had better clinical outcome than the other patients, even when limited to all RAS wild-type patients. CEA is a member of the immunoglobulin gene superfamily, and is overexpressed in about 90% of CRCs [21]. Serum CEA is used as a tumor marker for management of CRC [22]. On the other hand, Bhatnagar *et al.* noticed that in well differentiated CRC there is more production of CEA/gram of total protein than in the poorly differentiated tumors [23]. In our study, the proportion of patients with serum CEA level higher than 5.0 ng/ml among histological grade I/II tumor patients was significantly higher than those among histological grade III/IV tumor patients (68.0% versus 0%, respectively; Fisher test, $P = 0.0090$). The present study showed histological tumor grade of patients with high CEA level tended to be moderately/well differentiated, and these patients could experience good treatment response. However, further studies are needed, since this study was retrospective and limited by small sample size.

In Japan, the number of elderly people who need colorectal cancer chemotherapy has increased, as well as in the USA and Europe. Bevacizumab, a monoclonal antibody against vascular endothelial growth factor-A, was reported to be clinically useful for aged mCRC patients in the AVEX trial [24]. In contrast, the

cetuximab treatment for aged patients with mCRC is still considered to be debatable. The efficacy and adverse effects of the cetuximab-based medication for aged patients have not been thoroughly assessed. Our study included aged patients (over the age of 75), and compared them statistically against non-aged patients (under the age of 75). Among the aged patients, ORR was 43%, and DCR was 100% (data not shown). Median PFS and median OS were 7.3 months and 32.7 months, respectively. There were no significant differences at all in clinical outcomes between aged patients and non-aged patients. Incidence of adverse effects was also the same (data not shown). Cetuximab treatments for aged patients were not less efficacious compared with non-aged patients in some sub-analysis of other past studies. Our results in clinical practice also showed that cetuximab-based regimen was effective and tolerable for aged patients.

5. Conclusion

Good PS, left-sided CRC, high serum level of CEA, and well/moderately differentiated type showed associations with good clinical efficacy. ECOG PS, tumor location, histological grade, and serum CEA level at baseline might be useful predictors of cetuximab efficacy in the first-line treatment of mCRC.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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