

Research Article

Biomarker Analysis in A Randomized Phase 2 Study of Panitumumab Versus Cetuximab in Colorectal Cancer (WJOG6510GTR)

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Received: 06 October 2020; **Accepted:** 16 October 2020; **Published:** 27 October 2020

Citation: Hiroya Taniguchi, Yasuhiro Koh, Naotoshi Sugimono, Tomohiro Nishina, Takao Tamura, Hiroki Hara, Taito Esaki, Tadamichi Denda, Akitaka Makiyama, Aya Sakai, Hiroyuki Okuda, Naoki Izawa, Takayuki Ando, Kentaro Yamazaki, Shinya Tokunaga, Toshikazu Moriwaki, Akihito Tsuji, Hidekazu Kuramochi, Katsunori Shinozaki, Yukinori Ozaki, Hironori Yamaguchi, Hisateru Yasui, Satoshi Otsu, Mio Ikeda, Junji Kishimoto, Taroh Satoh, Daisuke Sakai, Kei Muro. Biomarker Analysis in A Randomized Phase 2 Study of Panitumumab Versus Cetuximab in Colorectal Cancer (WJOG6510GTR). *Journal of Cancer Science and Clinical Therapeutics* 4 (2020): 538-549.

Abstract

Background: The randomized phase II WJOG6510G study demonstrated the non-inferiority of panitumumab to cetuximab in terms of progression-free survival (PFS) in patients with wild-type *KRAS* exon 2 metastatic colorectal cancer. In this study, we performed exploratory analyses of updated survival data using the *KRAS* exon 2 and *RAS/BRAF* statuses.

Methods: Patients with wild-type *KRAS* exon 2 metastatic colorectal cancer who experienced progression after the

failure of fluoropyrimidine, oxaliplatin, and irinotecan were randomized to receive panitumumab or cetuximab in combination with irinotecan. An independent central laboratory performed *RAS/BRAF* testing using a PCR-reverse sequence-specific oligonucleotide method.

Results: In the updated analysis of 121 enrolled patients after a median follow-up of 31.3 months, the median PFS was 5.4 months in the panitumumab arm, versus 4.3 months in the cetuximab arm (hazard ratio [HR]=0.68). The median overall survival (OS) times were 14.9 and 12.5 months in

the panitumumab and cetuximab arms, respectively (HR=0.68). In 83 analyzed patients, *RAS/BRAF* testing identified 19 (23%) and 4 patients (5%) with *RAS* and *BRAF* mutations, respectively. In the wild-type *RAS* and *BRAF* population, trends of better PFS (6.5 months versus 4.6 months; HR=0.57) and OS (15.3 months versus 11.8 months; HR=0.77) were observed for panitumumab.

Conclusions: In this updated analysis, panitumumab was associated with a modest survival benefit versus cetuximab in patients with centrally confirmed wild-type *KRAS* exon 2 metastatic colorectal cancer as well as in the wild-type *RAS* and *BRAF* population.

Keywords: RAS; BRAF; Panitumumab; Cetuximab; Colorectal cancer

Abbreviations: ADCC- Antibody dependent cellular cytotoxicity; CI- Confidence interval; CRC- Colorectal cancer; ECOG PS- Eastern Cooperative Oncology Group performance status; EGFR- Epidermal growth factor receptor; Fc- Crystalline fragment; FcγRs- Fc gamma receptors; HR- Hazard ratio; OS- Overall survival; PFS- Progression-free survival; RECIST- The Response Evaluation Criteria In Solid Tumors

1. Introduction

KRAS and *NRAS* (*RAS*) mutations are present in approximately 50–55% of patients with colorectal cancer (CRC). *KRAS* exon 2 mutations have been already established as a negative predictive biomarker for anti-epidermal growth factor receptor (EGFR) antibody therapies such as cetuximab or panitumumab [1, 2]. Moreover, prospective and retrospective analyses in pivotal randomized clinical trials consistently revealed that anti-EGFR antibodies are unlikely to provide benefits in patients

with *KRAS* (exons 3 and 4) and *NRAS* mutations (exons 2, 3, and 4), as previously reported for patients with *KRAS* exon 2 mutations [3, 4]. On the basis of these results, *RAS* mutation testing is recommended prior to the initiation of anti-EGFR antibody therapy for patients with metastatic CRC in treatment guidelines to date [5].

BRAF is a serine-threonine kinase that is also located downstream of the EGFR pathway in the Ras/Raf/MAPK pathway. The V600E mutation is the most common *BRAF* mutation in CRC, being reported in 5–12% of patients with metastatic CRC, and *RAS* and *BRAF* V600E mutations are almost mutually exclusive. In addition, *BRAF* V600E mutations are more frequent in right-sided CRC than in left-sided CRC, and 30% of cases of *BRAF*-mutant CRC feature coexistent microsatellite instability [6]. Unlike *RAS* mutations, the predictive value of *BRAF* mutations regarding the efficacy of anti-EGFR therapy is less certain. Contrarily, the *BRAF* V600E mutation leads to a poor prognosis or rapid progression regardless of treatment in metastatic CRC [7, 8].

Antibody-dependent cellular cytotoxicity has been identified as a mechanism of cancer cell death. It is mediated by the bifunctional structure of IgG molecules. As a chimeric IgG1 monoclonal antibody, cetuximab has an antigen-binding fragment that may engage the cancer cell antigen and a crystalline fragment (Fc) that binds Fc gamma receptors (*FcγRs*) on effector cells. *FcγRs* are composed of three distinct classes, and several studies found that the efficacy of chemotherapeutic regimens including cetuximab is related to *FcγR* genotypes [9, 10].

Panitumumab is also an EGFR-directed antibody, but it is a fully human IgG2 monoclonal antibody, thereby differing from cetuximab. We previously reported the main results of

the WJOG6510G trial, which is a head-to-head, randomized phase 2 study of panitumumab versus cetuximab, both in combination with irinotecan, for use in patients with locally evaluated wild-type *KRAS* exon 2 metastatic CRC. The study found that panitumumab was associated with favorable progression-free survival (PFS) and overall survival (OS) compared with cetuximab. The median PFS was 5.4 months in the panitumumab arm versus 4.3 months in the cetuximab arm (hazard ratio [HR]=0.64; 95% confidence interval [CI]=0.44–0.94; $p < 0.001$ for non-inferiority, $p=0.06$ for superiority), and the median OS times in these groups were 14.9 and 11.5 months (HR=0.68; 95% CI=0.45–1.03; $p=0.05$), respectively [11].

In this study, we reported biomarker analyses of the WJOG6510G study to determine whether centrally evaluated extended *RAS* and *BRAF* mutations and *FcγR* polymorphisms are related to the efficacy of cetuximab and panitumumab in combination with irinotecan.

2. Materials and Methods

2.1 Patients

This WJOG6510GTR trial is an accompanying biomarker study (UMIN000008786) of WJOG6510G (UMIN000006643). Concerning the WJOG6510G trial, detailed information regarding patient inclusion criteria, study design, and treatment schedules was previously reported. Briefly, the major eligible criteria were as follows: histologically confirmed unresectable metastatic CRC; lesions that are refractory or intolerant to fluorouracil-, oxaliplatin-, and irinotecan-based chemotherapy; wild-type *KRAS* exon 2 based on the local assessment; Eastern Cooperative Oncology Group performance status (ECOG PS) of 0–2; presence of measurable disease as defined by the Response Evaluation Criteria In Solid Tumors (RECIST) v1.1; and adequate hematologic, renal, hepatic,

and metabolic function. Patients previously treated with an anti-EGFR antibody were excluded. These trials were conducted according to the ethical principles of the Declaration of Helsinki, and the study protocol was approved by the institutional review board of the participating institutions. All patients provided written informed consent.

2.2 Treatment and efficacy assessment

Patients were randomized on a 1:1 basis to receive panitumumab (6 mg/kg) intravenously on day 1 of each 14-day cycle or cetuximab at an initial intravenous dose of 400 mg/m² followed by 250 mg/m² intravenously on day 1 of each 7-day cycle. Irinotecan at 150 mg/m² was administered intravenously in combination with panitumumab or cetuximab every 2 weeks. The starting dose of irinotecan could be reduced to 100 or 120 mg/m² in patients who required dose reduction during prior treatment. Treatment continued until disease progression, intolerability, or withdrawal of consent. For efficacy assessment, tumor assessments were repeated every 8 weeks from randomization to discontinuation of the protocol treatment. Responses were assessed by each investigator according to RECIST v1.1, and there was no central review of response. Laboratory assessments were assessed at screening, baseline, and then once every 2 weeks thereafter.

2.3 RAS and BRAF mutation analysis

In this biomarker analysis, *RAS* and *BRAF* mutations were centrally assessed in a central laboratory (G&G Science Co., Ltd., Fukushima, Japan). Genomic DNA was extracted from formalin-fixed, paraffin-embedded CRC tissues obtained from biopsies or surgical specimens. Extracted DNA samples were diluted to a concentration of 10–20 ng/μL using sterile TE buffer (1 mmol/L Tris-HCL

[pH 8.0], 0.1 mmol/L EDTA). Assays were performed according to the manufacturer's protocol using a bead-based multiplexed immunoassay system (xMAP technology; Luminex). Mutations in *KRAS* and *NRAS* codons 12, 13, 59, 61, 117, and 146 were detected using the PCR-based MEBGEN RASKET kit (MBL, Japan) [12]. We also used the Genosearch BRAF kit to detect *BRAF* V600 mutations (V600E, V600K, V600R, and V600D) and mutations such as those in exon 11, including mutations in codons 464 (G464E, G464V, and G464R), 466 (G466R, G466V, and G466E), 467 (S467L), 469 (G469A, G469V, G469R, and G469E), 485 (L485F), 524 (Q524L), 525 (L525R), 581 (N581S, N581I, and N581T), 594 (D594N and D594G), 596 (D596R), 597 (L597R, L597S, L597V, L597Q, and L597P), 598 (A598T), 599 (T599_600insT), and 601 (V601E and V601N) [13].

2.4 Examination of FcγR genotyping

Samples were collected in blood collection tubes containing ethylenediaminetetraacetic acid disodium salt as an anticoagulant, and genomic DNA samples were extracted using a QIAamp DNA Mini kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. The genotyping of *FcγR2A* H131R (rs180127), *FcγR3A* V158F (rs396991), and *FcγR2B* I232T (rs1050501) polymorphisms was conducted using TaqMan SNP Genotyping Assays C_9077561_20 and C_25815666_10 and the custom-designed assay AHHS8M6 (Applied Biosystems, Foster City, CA, USA), respectively, according to the manufacturer's protocols with modifications.

2.5 Statistical analysis

Fisher's exact test and a trend test were used to calculate P values for the associations between genetic parameters and treatment efficacy. OS was defined as the time from registration to death from any cause. PFS was defined as

the time from registration to the first appearance of progression or death from any cause. The associations of patient characteristics with OS were examined using Cox proportional hazards regression models. Data are reported as HR estimates with their 95% CIs. The associations of genetic parameters with OS and PFS were analyzed using Kaplan–Meier curves and the log-rank test. All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R 2.13.0 (R Foundation for Statistical Computing, Vienna, Austria) [14]. More precisely, EZR is a modified version of R commander (version 1.6-3) that includes statistical functions used frequently in biostatistics. All P values were two-sided, and $P < 0.05$ denoted statistical significance.

3. Results

3.1 Patients and updated efficacy

In the WJOG6510G trial, a total of 121 patients were enrolled between December 2011 and September 2014, and 1 patient was excluded from the full analysis set (FAS) because of ineligibility. Therefore, the FAS included 59 patients in the cetuximab arm and 61 patients in the panitumumab arm (Supplemental Figure 1). The cutoff date for the initial primary analysis was September 2015, whereas that for the updated analysis was March 2017. The median follow-up times for the analyses were 35.1 and 32.7 months in the cetuximab and panitumumab arms, respectively. Patient and disease characteristics were well balanced between the two arms (Table 1). The median patient age was 64 years, and >85% of patients had left-sided CRC. At the time of the updated analysis, all FAS patients were evaluable for efficacy. Of these patients, 117 patients (97.5%) experienced disease progression, and 113 patients (94.2%) died. Concerning PFS, which is the primary endpoint of the WJOG6510G trial, a significant

benefit in favor of panitumumab was observed (HR=0.68; 95% CI=0.46–0.98; *p*=0.035; Figure 1a). The median PFS was 4.3 months in the cetuximab arm, versus 5.4 months in the panitumumab arm. A similar trend was also observed

for OS, as indicated by median OS times of 12.5 and 14.9 months in the panitumumab and cetuximab groups (HR=0.68; 95% CI=0.47–0.98; *p*=0.037; Figure 1b), respectively.

		Whole population		RAS/BRAF wild-type	
		Cetuximab (n=59)	Panitumumab (n=61)	Cetuximab (n=32)	Panitumumab (n=30)
Age (years), median (range)		64 (33-79)	64 (41-80)	63.5 (33-75)	66 (41-79)
Sex	Male	37 (62.7%)	42 (68.9%)	21 (65.6%)	19 (63.3%)
	Female	22 (37.3%)	19 (31.1%)	11 (34.4%)	11 (36.7%)
ECOG PS	0	32 (54.2%)	38 (62.3%)	17 (53.1%)	19 (63.3%)
	1	26 (44.1%)	22 (36.1%)	14 (43.8%)	11 (36.7%)
	2	1 (1.7%)	1 (1.6%)	1 (3.1%)	0 (0%)
Tumor location ^b	Right sided	7 (11.9%)	9 (14.8%)	3 (9.4%)	4 (13.3%)
	Left sided	52 (88.1%)	52 (85.2%)	29 (90.6%)	26 (86.7%)
Primary tumor location	Present	10 (16.9%)	11 (18.0%)	5 (15.6%)	5 (16.7%)
	Resected	49 (83.1%)	50 (82.0%)	27 (84.4%)	25 (83.3%)
Histology	Low grade	3 (5.1%)	6 (9.8%)	1 (3.2%)	2 (6.7%)
	High grade	56 (94.9%)	55 (90.2%)	31 (96.8%)	28 (93.3%)
Number of metastatic sites	1	20 (33.9%)	30 (49.1%)	9 (28.1%) ^a	13 (43.3%)
	≥2	39 (66.1%)	31 (50.9%)	22 (67.8%)	17 (56.7%)
Time from starting first-line chemotherapy	≤18 months	28 (47.4%)	18 (29.5%)	18 (56.2%)	9 (30.0%)
	>18 months	31 (52.6%)	43 (70.5%)	14 (43.8%)	21 (70.0%)

^a One patient had unresectable colorectal cancer without a metastatic site. ^b Primary tumors proximal to the splenic flexure were considered right-sided tumors, and those at or distal to the splenic flexure were considered left-sided tumors. ECOS PS, Eastern Cooperative Oncology Group performance status

Table 1: Patients’ backgrounds.

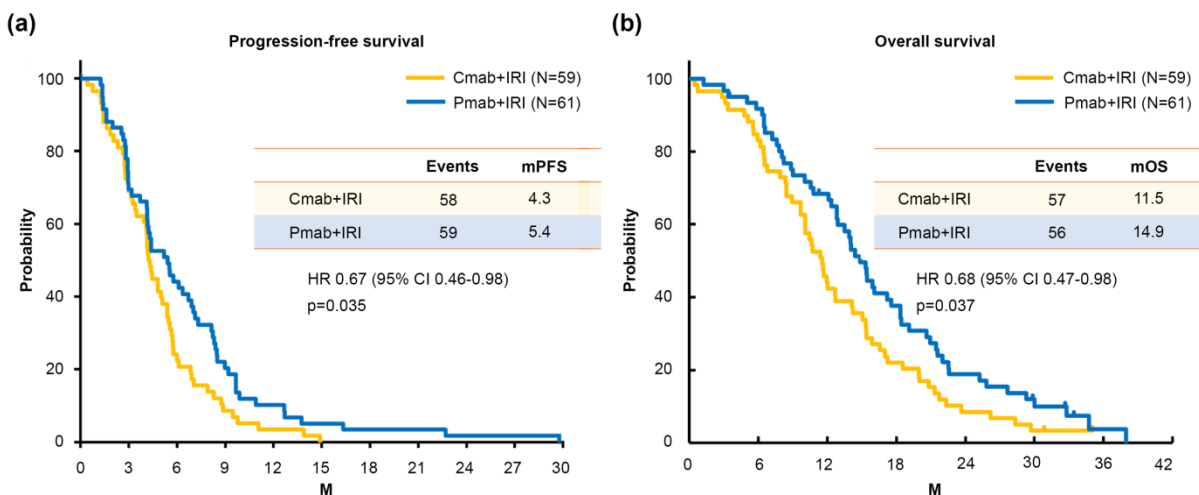


Figure 1: Updated survival outcomes in the whole population. Cmam, cetuximab; Pmam, panitumumab; IRI, irinotecan; HR, hazard ratio; CI, confidence interval; mPFS, median progression-free survival; mOS, medial overall survival; M, months

3.2 Efficacy according to the RAS and BRAF mutation status

Of the 120 patients included in the WJOG6510G study, the *RAS* and *BRAF* mutation status was evaluable in 86 patients (71.7%; Supplemental Figure 2). Samples from the remaining 34 patients were not available because of uncollectible tumors, insufficient residual tumors, or failure of the assay. Of the evaluable tumors, *RAS* mutations were identified in 19 tumors, including 9 tumors with *KRAS* exon 2 mutations. *BRAF* mutations were found in five patients, including the V600E mutation in three patients. All *RAS* and *BRAF* mutations were mutually exclusive in this study; therefore, 62 tumors were deemed wild-type for *RAS* and *BRAF*. In the survival analysis, clearly better survival was demonstrated in patients with wild-type *RAS* and *BRAF* tumors than in those with *RAS*- or *BRAF*-mutant tumors (Table 2). No patients with *RAS*-mutant or *BRAF*-mutant

tumors responded to treatment, excluding one patient with a *BRAF* G466E mutation who responded to panitumumab plus irinotecan (Supplemental Table 1 and Supplemental Figure 3).

Comparing the cetuximab and panitumumab arms in the *RAS* and *BRAF* wild-type population, no differences in patient background were observed between the two arms. A clear and significant benefit associated with panitumumab treatment versus cetuximab was apparent for PFS (median, 6.5 months versus 4.6 months; HR=0.57; 95% CI=0.34–0.98; $p=0.038$; Figure 2a). In terms of OS, the panitumumab arm displayed numerically better survival without reaching significance (median, 15.3 months versus 11.8 months; HR=0.77; 95% CI=0.45–1.30; $p=0.32$; Figure 2b).

		<i>RAS/BRAF</i> wild (n=62)	<i>RAS</i> mutant (n=19)	<i>BRAF</i> mutant (n=5)	p value
Progression-free survival	Number of events	62	19	5	p=0.0013
	Median (months)	5.3	2.8	4.4	
Overall survival	Number of events	58	19	5	p=0.11
	Median (months)	14	9.7	8.8	

Table 2: Survival outcomes according to RAS/BRAF status.

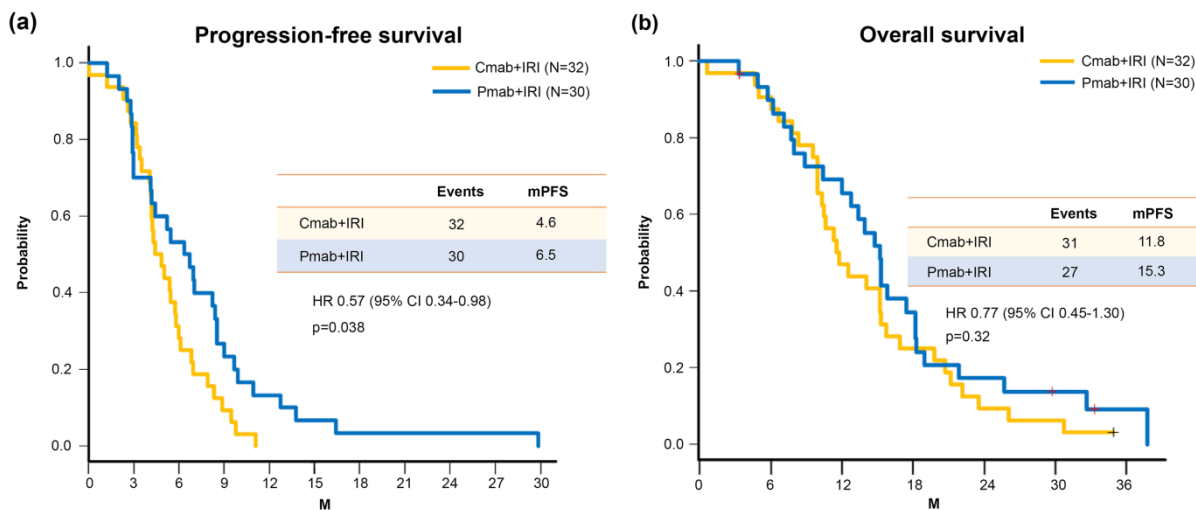


Figure 2: Updated survival outcomes in the wild-type RAS/BRAF population. Cmab, cetuximab; Pmab, panitumumab; IRI, irinotecan; HR, hazard ratio; CI, confidence interval; mPFS, median progression-free survival; mOS, medial overall survival; M, months

3.3 Analysis of FcγR polymorphisms

The analysis of *FcγR* polymorphisms could only be performed in 36 patients (30.0%). The *FcγR2A* distribution did not concur with the expected distribution (HH, 66.7%; HR, 27.8%; RR, 5.6%). The frequencies of *FcγR3A* polymorphisms for VV, VF, and FF were 8.3%, 38.9%, and 41.2%, respectively (Supplemental Table 2). Four patients were not categorized because of existing copy number

variation for *FcγR3A*. In the analysis of the relationships between *FcγR* polymorphisms and response rate, no clear difference was noted between the panitumumab and cetuximab arms (Supplemental Table 3). *FcγR2B* polymorphisms for II, IT, and TT were found in 69.4%, 27.8%, and 2.8%, respectively, of patients, and no clear relationship to treatment efficacy was observed.

4. Discussion

The aim of our post hoc analysis of the WJOG6510G study was to investigate the effects of *RAS* and *BRAF* mutations on treatment efficacy. In the evaluable patients with wild-type *RAS* and *BRAF* tumors, better survival outcomes were observed in the panitumumab arm, in line with the results for the FAS population in the original report for WJOG6510G. As discussed in the original paper, surprisingly better PFS and OS were observed in the panitumumab arm. Although the mechanisms of these findings are unclear, possible explanations include prior bevacizumab exposure. For example, panitumumab has greater affinity for EGFR than cetuximab, permitting the former drug to bind the receptor under hypoxic conditions after bevacizumab therapy. A subgroup analysis of the ASPECCT trial, which was also a head-to-head comparison of panitumumab and cetuximab monotherapy [15], also reported trends of better PFS and OS in favor of panitumumab in patients who previously received bevacizumab [16]. The true mechanism remains unknown, but the *RAS/BRAF* analysis in this paper supports that the imbalance of patients with *RAS*-mutant tumors does not explain the difference in outcome in the WJOG6510G trial.

The method of *RAS* testing sometimes affects the results for the *RAS* mutation status. In fact, we identified nine patients with discordant results between the local and central assessment of the *KRAS* exon 2 status. For central *RAS* testing, we selected the PCR-based MEBGEN™ RASKET assay with a detection limit of at least 1–5%, which was better than that of the direct sequencing method used for local assessment. In total, we newly identified 19 patients with *RAS*-mutant tumors, none of whom responded to anti-EGFR antibody plus irinotecan therapy (data not shown). Although the significance of low-prevalence *KRAS* or *RAS* mutations in relation to the effectiveness of anti-EGFR

antibody therapy in metastatic CRC remains unclear [17, 18], this PCR-based assay is appropriate for selecting patients with anti-EGFR therapy considering our results. To date, the MEBGEN™ RASKET-B kit, which can detect 48 types of *RAS* mutations and the *BRAF* V600E mutation using the same method as the RASKET kit in the present study, is approved and widely used in Japanese clinical practice [19].

The effects of *BRAF* mutations on the efficacy of anti-EGFR therapy are also controversial. In this study, only 3/86 (3.5%) wild-type *KRAS* exon 2 tumors harbored the *BRAF* V600E mutation. Ethnic differences in the distribution of the *BRAF* V600E mutation between Western countries (8–15%) and Japan (approximately 5%) have been reported [20], but the low prevalence in this study was mainly attributable to the poor prognosis of patients carrying the *BRAF* V600E mutation. This study included patients who previously received two or more lines of therapy; therefore, it is difficult to enroll patients with the *BRAF* V600E mutation and aggressive tumors in later-line clinical trials such as this study. Although clinical trials in the first-line setting demonstrated the benefits adding anti-EGFR antibody even among patients with *BRAF* V600E tumors [21], these tumors hardly responded to anti-EGFR therapy in later lines [22], in line with our findings that none of three patients with the *BRAF* V600E mutation responded to the combination of an anti-EGFR antibody plus irinotecan.

Differing from the findings in patients carrying the *BRAF* V600E mutation, some patients with *BRAF* non-V600E mutations respond to anti-EGFR therapy. Yao et al. classified *BRAF* mutations into three groups based on their biochemical and signaling mechanisms [23]. Among these, class 3 *BRAF* mutants exhibit low kinase activity or kinase

death, but they activate the MAPK pathway through enhanced RAS binding and subsequent RAS-dependent CRAF activation, which could render them sensitive to anti-EGFR therapy [24]. In line with this previous report, one patient with the *BRAF* G469 mutation in our cohort responded to panitumumab plus irinotecan therapy. When comprehensive genomic profiling using next-generation sequencing becomes a standard of care in patients with metastatic CRC, more patients with *BRAF* non-V600E mutations will be observed in daily clinical practice. At present, we will continue to apply anti-EGFR therapy according to the *BRAF* non-V600E classification.

We found no significant association between *FcγR* polymorphisms and the clinical response to cetuximab and panitumumab treatment in this study. We had assumed that polymorphisms, especially those in *FcγR2A* and/or *FcγR3A*, are predictive biomarkers between cetuximab and panitumumab before starting enrollment in this study because cetuximab, which is a chimeric IgG1 antibody, has greater potential to induce antibody dependent cellular cytotoxicity (ADCC) than panitumumab, which is a fully human IgG2 antibody. Some previous studies found that the *FcγR2A* 131H/H and *FcγR3A* 158F/F genotypes are associated with longer PFS, although another study found no association of *FcγR2A* or *FcγR3A* polymorphisms with survival, in line with our study [25]. Although we could analyze *FcγR* polymorphisms in only 38 patients, all 3 patients with *FcγR2A* 131H/R and *FcγR3A* 158F/V polymorphisms exhibited partial responses to treatment with panitumumab, which was not expected to induce ADCC.

Because of the relatively small sample sizes in the *RAS*, *BRAF*, and *FcγR* analyses, there were risks of imbalances in important prognostic factors despite patient randomization

into the treatment arms. For this reason, we should consider potential differences in important baseline characteristics. To date, ECOG PS and tumor location are considered powerful prognostic factors for anti-EGFR therapy [26]. In this study, no clear difference in survival was observed between the study arms even though nearly 90% of patients had left-sided tumors, which were expected to have greater responses to anti-EGFR therapy than right-sided tumors. We did not adjust for these prognostic factors because of the small sample sizes, but consideration of these factors would not change the results.

5. Conclusions

In conclusion, our study supports the benefits of panitumumab plus irinotecan versus cetuximab plus irinotecan in the treatment of metastatic CRC in patients with wild-type *RAS* tumors. On the basis of the lack of an observed response, anti-EGFR therapy is contraindicated for patients with *RAS*-mutant tumors. The benefits of anti-EGFR therapy for *BRAF* V600E mutant tumors may be very small or nonexistent, whereas substantial benefits were observed in patients with *BRAF* non-V600E mutant tumors. However, further data are required to clarify these observations.

Acknowledgments

We thank the patients, investigators, and institutions involved in this study. We also thank the data managers and other support staff of the West Japan Oncology Group (WJOG), especially Dr. Shinichiro Nakamura and Kaori Mori.

Funding

This study was supported by WJOG, a non-profit organization, and funded by WJOG Funds.

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