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A Multicenter Retrospective Study on the Clinical Features of Patients with Human Epidermal Growth Factor Receptor 2-Positive Colorectal Cancer (HGCSG2304)

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Abstract

Background

In metastatic colorectal cancer (mCRC), human epidermal growth factor receptor 2 (HER2)-positive disease is an important molecular subtype for targeted therapy; however, its prevalence is low and real-world data remain limited.

Methods

A multicenter retrospective study of patients with HER2-positive mCRC diagnosed between 2010 and 2023 at 14 institutions in Japan was conducted. In patients with *RAS* wild-type tumors, clinical outcomes were compared based on the molecular targeted agent (anti- epidermal growth factor receptor [EGFR] antibody or bevacizumab) combined with first-line chemotherapy. In patients treated with trastuzumab plus pertuzumab, clinical outcomes and safety including infusion-related reactions (IRRs) were assessed.

Results

Forty-five patients were included. In patients with *RAS* wild-type tumors, clinical outcomes were comparable between the anti-EGFR antibody (n = 17) and bevacizumab (n = 9) groups (progression-free survival: 15.6 *vs*. 12.0 months; hazard ratio: 0.94, 95% confidence interval: 0.38–2.35, overall survival: 38.4 *vs*. 32.0 months; hazard ratio: 0.91, 95% confidence interval: 0.30–2.74, p = 0.87). Twenty patients received trastuzumab plus pertuzumab, with a median progression-free survival of 3.1 months and an objective response rate of 10%. Greater benefit was observed in patients with *RAS* wild-type and HER2 immunohistochemistry
3+ tumors (objective response rate 28.6%). IRRs occurred in 14.2% of patients who received prophylactic antihistamines and 41.7% of those who did not.

Conclusions

In patients with HER2-positive mCRC, first-line treatment efficacy appeared comparable between the anti-EGFR antibody and bevacizumab groups. Trastuzumab plus pertuzumab demonstrated clinical activity, emphasizing the importance of appropriate patient selection and management of IRRs.

Introduction

Metastatic colorectal cancer (mCRC) remains one of the leading causes of cancer-related morbidity and mortality worldwide [1]. In mCRC, recent advances in the development of molecular target agents have enabled treatment stratification based on specific molecular subtypes, including distinct genetic alterations. Among these, human epidermal growth factor receptor 2 (HER2)-positive mCRC is a particularly clinically significant subtype due to the efficacy of HER2-targeted therapies, such as the combination of trastuzumab plus pertuzumab (Tmab+Per) in the MyPathway and the TRIUMPH studies

[2, 3], and trastuzumab deruxtecan in the DESTINY-CRC 01 and DESTINY-CRC02 trials [4, 5]. However, HER2-positive disease occurs in only approximately 3–5% of all mCRC cases [6, 7], so real-world data of clinical characteristics, optimal treatment strategies, and outcomes of HER2-targeted therapy remain limited, leaving several clinically important questions unresolved.

First, it is unclear whether an anti-epidermal growth factor receptor (EGFR) antibody or bevacizumab is the optimal molecular targeted agent as first-line treatment for *RAS* wild-type HER2-positive mCRC patients, who reportedly constitute the majority of HER2-positive colorectal cancer cases. Currently, both molecular targeted agents are available for first-line treatment of RAS wild -type mCRC. However, multiple studies have reported that anti-EGFR antibody therapy is less effective in later-line treatment of HER2-positive mCRC [6, 8, 9]. Indeed baseline circulating tumor DNA analysis [10] in the PARADIGM trial demonstrated higher efficacy of anti-EGFR agents in population without multiple genetic alterations, including *HER2* amplification. Consequently, there is no established consensus on the optimal molecular targeted agent for first-line treatment in patients with *RAS* wild-type HER2-positive mCRC.

Second, there is a lack of real-world data on the efficacy and safety of Tmab+Per combination therapy, which is currently the most widely used HER2-targeted regimen in clinical practice. The objective response rate (ORR) of this combination in clinical trials is approximately 20–30% [2, 3], but the data from real-world practice remain limited, making it important to investigate which patients are most likely to benefit. Furthermore, infusion-related reactions (IRRs) have been reported particularly during early treatment cycles [3, 11], but information on the strategies for safe administration in routine clinical practice remains insufficient. Therefore, we conducted a multicenter retrospective observational study to address these clinical questions in patients with HER2-positive mCRC.

Patients and Methods

Patients, Study Design, and Definition of Human Epidermal Growth Factor Receptor 2-Positivity

This multicenter retrospective observational study included patients diagnosed with HER2-positive mCRC at 14 institutions in Japan between December 2010 and December 2023. HER2-positive tumors were defined by at least one of the following criteria: i) an immunohistochemistry (IHC) score of 3+, ii) a HER2/CEP17 ratio of 2.0 or greater by fluorescence in situ hybridization (FISH), and iii) HER2 gene amplification detected by next-generation sequencing (NGS) using tissue or liquid biopsy, defined as a copy number of 2.18 or more.

RAS/BRAF Mutation and Microsatellite Instability/Mismatch Repair Status

RAS and BRAF V600E mutations were analyzed using the RASKET-B kit (Medical & Biological Laboratories, Tokyo, Japan). Microsatellite instability (MSI) status was determined either by a polymerase chain reaction-based MSI panel or by IHC staining for mismatch repair proteins (MutL homolog 1, MutS homolog 2, MutS homolog 6, and Postmeiotic segregation increased 2).

Treatment Outcomes

In patients with RAS wild-type tumors, the efficacy of first-line doublet or triplet chemotherapy combined with either anti-EGFR antibody or bevacizumab was evaluated. Progression-free survival (PFS) was defined as the time from initiation of first-line therapy to disease progression or death from any cause. Overall survival (OS) was defined as the time from initiation of first-line therapy to death from any cause. ORR was assessed according to the Response Evaluation Criteria in solid tumor version 1.1. The efficacy and safety of Tmab+Per were analyzed in patients who initiated this regimen by July 2024. ORR, disease control rate (DCR), and PFS were assessed according to the Response Evaluation Criteria in solid tumor version 1.1. Adverse events (AEs) were evaluated using the Common Terminology Criteria for Adverse Events version 5.0. The data cutoff date was December 31, 2025.

Statistical Analysis

Statistical analyses were performed using EZR version 1.68 (Saitama Medical Center, Jichi Medical University, Saitama, Japan) and IBM SPSS Statistics version 29.0.1.0 (IBM Japan Ltd., Tokyo, Japan). Categorical variables were evaluated using Fisher's exact test. Survival curves were estimated using the Kaplan–Meier method, and hazard ratios (HRs) were calculated using the Cox proportional hazards model. Comparison of PFS between treatment groups was performed using the log-rank test. Statistical significance was defined as a *p*-value less than 0.05.

Ethical Considerations

This study was conducted in accordance with the Declaration of Helsinki and approved by the institutional review boards of all participating institutions. Due to the retrospective nature of the study, the requirement for written informed consent was waived.

Results

Baseline Characteristics of Study Participants

A total of 45 patients with HER2-positive mCRC were included in this study. Patient clinical characteristics for all 45 patients are shown in Table 1. The median age was 63 years (range, 36–81 years), and 64.4% were male. HER2 status was determined as follows: 26 patients had HER2 IHC3+, 15 patients were FISH positive (excluding IHC3+), and 4 patients had *HER2* gene amplification detected by NGS (excluding IHC3+ and FISH positive).

Primary tumors were located on the left side of the colon or rectum in 73.3% of patients and on the right side in 26.7%. Metastatic sites were most commonly the liver (66.7%), followed by the lung (35.6%). Regarding molecular profiles, 73.3% of patients were *RAS* wild-type, and all patients were negative for the *BRAF* V600E mutation and MSI-high (MSI-H) status. Detailed results of HER2 testing by IHC, FISH, and NGS, along with corresponding *RAS* status for each patient, are summarized in Supplementary Table 1.

Of 33 patients with *RAS* wild-type tumors, nine received anti-EGFR and seventeen received bevacizumab combined with chemotherapy as first-line treatment (anti-EGFR antibody *vs*. bevacizumab cohort). Twenty of the 45 patients received Tmab+Per therapy (Tmab+Per cohort). The study flowchart is shown in Figure 1.

Efficacy of First-Line Therapy in RAS Wild-Type Patients

Patient characteristics for those with HER2-positive and *RAS* wild-type tumors who received first-line chemotherapy with an anti-EGFR antibody (*n* = 17) or bevacizumab (*n* = 9) are shown in Supplementary Table 2. PFS and OS according to first-line regimens in patients with HER2 positive/*RAS* wild-type are shown in Figures 2A and 2B. At a median follow-up of 43.3 months, first-line PFS was similar between the anti-EGFR antibody (*n* = 17) and bevacizumab (*n* = 9) groups (median: 15.6 *vs*. 12.0 months, HR = 0.94, 95% confidence interval: 0.38–2.35, *p* = 0.89). OS was also similar between the two groups (median: 38.4 *vs*. 32.0 months; HR = 0.91, 95% confidence interval: 0.30–2.74, *p* = 0.87). The ORR was 58.8% in the anti-EGFR antibody group and 100% in the bevacizumab group, with details provided in Supplementary Table 3.

Efficacy and Safety Profile of Trastuzumab plus Pertuzumab Therapy

A total of twenty patients received Tmab+Per therapy. Baseline characteristics of these patients are summarized in Table 2. The median age was 65 years (range, 36–80), with 9 females (45.0%) and 11 males (55.0%). Primary tumors were located more frequently on the left side of the colon or rectum (75.0%) than on the right side (25.0%). Regarding HER2 status, 9 patients (45.0%) had IHC 3+, 7 patients (35.0%) were FISH positive (excluding IHC 3+), and 4 patients (20.0%) had HER2 gene amplification confirmed by NGS (excluding IHC 3+ and FISH positive). RAS wild-type status was identified in 15 patients (75.0%), while 5 patients (25.0%) carried RAS mutations. Tmab+Per therapy was administered mainly in later treatment lines; 4 patients (20.0%) as second-line, 1 patient (5.0%) as third-line, 7 patients (35.0%) as fourth-line, 5 patients (25.0%) as fifth-line, and 3 patients (15.0%) as sixth-line therapy. Median relative dose intensity was 97.9% for trastuzumab and 97.7% for pertuzumab, indicating good treatment compliance. Figure 3 shows the PFS for Tmab+Per therapy, along with each patient's HER2-IHC score, RAS status, prior anti-EGFR therapy, and line of therapy. Median PFS for all patients was 3.1 months. The ORR and DCR were 10.0% and 55.0%, respectively. Efficacy by RAS status and HER2 IHC score is shown in Figure 4. Patients most likely to benefit from Tmab+Per treatment were those with RAS wild-type and IHC3+ tumors (median PFS 4.1 months, ORR 28.6%, DCR 71.4%), followed by RAS wild-type without IHC3+ (median PFS 2.2 months, ORR 0%, DCR 37.5%). Among five patients with RAS mutations, only one achieved disease control, with the poorest treatment response (median PFS 2.2 months).

AEs related to Tmab+Per therapy were generally manageable (Table 3). The most frequently observed AEs (any grade) included fatigue (40.0%), anorexia (30.0%), anemia (30.0%), and IRRs (30.0%). Grade 3 or more AEs occurred in 15% of patients and included diarrhea (5.0%), IRRs (5.0%), perimandibular

inflammation (5.0%), cellulitis (5.0%), and ureteral stones (5.0%). One patient discontinued Tmab+Per therapy due to Grade 4 IRRs.

The frequency of IRRs according to premedication is shown in Table 4. Among 19 patients with available premedication data, IRRs occurred in six patients (three Grade 1, two Grade 2, and one Grade 4). The incidence of IRRs was similar in patients who received acetaminophen or nonsteroidal anti-inflammatory drugs as premedication (n = 9) compared with those who did not (n = 11) (33.0% vs. 30.0%, p = 1.00). IRRs were numerically lower in patients who received antihistamines (n = 7) compared with those who did not (n = 12), although the difference was not statistically significant (14.2% vs. 41.7%, p = 0.35).

Discussion

In this multicenter retrospective study, we investigated the real-world clinical characteristics and treatment outcomes of patients with HER2-positive mCRC. To our knowledge, this study represents the largest multicenter real-world clinical cohort to date. Our data provide unique insights into the clinical characteristics, treatment outcomes, and premedication strategies of IRRs in this rare molecular subtype in routine practice.

The majority of patients in our cohort had left-sided primary tumors with *RAS* wild-type status and liver metastases, consistent with previous reports [6, 12]. Prior studies have shown that *HER2* amplification is largely mutually exclusive with MSI-H [12], and no patients in this study harbored MSI-H tumors or *BRAF* V600E mutations. Furthermore, compared with prior clinical evidence [3], our real-world cohort included a relatively higher proportion of patients defined as HER2-positive by FISH or NGS despite the absence of HER2IHC3+ overexpression. In prospective clinical trials such as DESTINY-CRC01 and TRIUMPH, approximately 75% of patients were classified as having HER2 IHC3+ tumors [3] [4], whereas only 57.8% (26/45) of patients exhibited HER2 IHC3+ overexpression in our study. Because IHC results are not mandatory for the use of anti-HER2 therapy in clinical practice, unlike clinical trials, the presence of a few patients (*n* = 4, 8.9%) with missing IHC results contributed to this difference. However, the primary reason was the high number of cases showing *HER2* gene amplification despite IHC2+ (*n* = 13, 28.9%). HER2 IHC in our study was performed using the same antibody clone (VENTANA 4B5) as in prospective trials; however, scoring was conducted locally at each participating institution rather than *via* centralized review. This difference in assessment methodology may have introduced inter-observer variability and partly explains the discrepancy in HER2 expression profiles between our real-world cohort and clinical trial populations. These findings suggest that when tumors are classified as HER2 IHC2+ by local assessment, confirmatory testing with FISH or NGS may help identify additional tumors with *HER2* gene amplification.

In first-line chemotherapy for patients with *RAS* wild-type tumors, there were no statistically significant differences in efficacy between the bevacizumab-containing and anti-EGFR antibody groups. Although HER2 overexpression has been proposed as a mechanism of resistance to anti-EGFR therapy based on preclinical and retrospective late-line treatment data [13], a recent pooled exploratory analysis of eight

randomized trials demonstrated that HER2 positivity did not predict differential benefit from first-line chemotherapy combined with either anti-EGFR antibodies or bevacizumab [14]. In that analysis, no significant interaction between HER2 status and the choice of biologic agent was observed for PFS, OS, or ORR, indicating that HER2 positivity alone should not guide selection between anti-EGFR- or bevacizumab-based regimens in the first-line setting. Therefore, the lack of significant differences observed in our study aligns with these findings. Clinically, these results support an individualized treatment approach in which the selection of biologic agents is guided primarily by patient-specific factors rather than expected differences in efficacy. In particular, the distinct toxicity profiles of anti-EGFR antibodies (e.g., skin toxicity) and bevacizumab (e.g., hypertension and proteinuria) should be carefully considered when choosing the most appropriate targeted agent for each patient.

In the present study, among patients treated with Tmab+Per, the ORR was 10.0%, DCR was 55.0%, and median PFS was 3.1 months. Compared with previously reported prospective studies, such as the MyPathway and TRIUMPH trials [2] [3], median PFS was generally comparable, whereas the ORR was lower in our cohort. Several differences in patient characteristics may explain this discrepancy. Compared with prior clinical trials, patients treated with Tmab+Per in our study were older, had a higher proportion of *RAS*-mutant tumors, and showed a lower frequency of HER2 IHC3+ expression. Among these factors, *RAS* mutation status and HER2 IHC score were particularly important. As anti-HER2 therapy is known to be less effective in *RAS*-mutant tumors, in the MyPathway study, Tmab+Per demonstrated substantially lower activity in patients with *RAS*-mutant tumors (ORR 8%) compared with patients with *RAS* wild-type disease (ORR 40%) [2]. The importance of HER2 expression level for anti-HER2 therapy has been demonstrated in the DESTINY-CRC01 trial evaluating trastuzumab deruxtecan, in which patients with HER2 IHC3+ tumors achieved higher ORR (57.5%) and longer median PFS (8.3 months), whereas limited benefit was observed in patients with HER2 IHC2+/FISH positive tumors (ORR 7.7%; median PFS 4.1 months) [4]. Consistently, in our study, treatment efficacy was highest in patients with *RAS* wild-type and IHC3+ tumors. Taken together, these findings indicate that careful patient selection based on molecular characteristics, including *RAS* mutation status and HER2 expression level, is crucial to maximize the benefit of late-line treatment, including anti-HER2 therapy, in metastatic colorectal cancer.

AEs associated with Tmab+Per were generally manageable, suggesting that this combination therapy can be administered with an acceptable safety profile in real-world clinical practice. In the TRIUMPH trial, IRRs, a characteristic AE of Tmab+Per therapy, were reported in 47.0% of patients [3]. IRRs are clinically relevant because their occurrence may lead to treatment interruption or delay, potentially compromising treatment continuity. Therefore, appropriate management and prevention of IRRs are important in clinical practice. In our real-world cohort, IRRs were observed in 31.6% of patients, demonstrating an incidence comparable to that reported in clinical trials. Notably, patients who received antihistamine premedication experienced a numerically lower incidence of IRRs compared with those who did not receive antihistamines (14.2% *vs*. 41.7%). In clinical practice, premedication with antihistamines or nonsteroidal anti-inflammatory drugs is commonly used to prevent IRRs associated with chimeric monoclonal antibodies such as rituximab [15, 16]. In breast cancer, corticosteroid premedication has also been

suggested to reduce the risk of IRRs during trastuzumab therapy in retrospective studies [17]. However, there are currently no prospective or retrospective studies that have specifically evaluated the efficacy of premedication, including corticosteroids or antihistamines, for the prevention of IRRs associated with trastuzumab or pertuzumab therapy in patients with metastatic colorectal cancer. Our findings suggest that antihistamine premedication may reduce the risk of IRRs in this setting.

Despite the clinical relevance of these real-world findings, several limitations should be acknowledged. First, this study was limited by its retrospective observational design, which may have resulted in incomplete clinical data and variability in the timing of response assessment and AE reporting. Second, the relatively small sample size, reflecting the rarity of HER2-positive mCRC, may have limited the statistical power to detect differences between treatment groups. Third, there was substantial heterogeneity in patient characteristics and treatment strategies. In particular, the line of therapy at which Tmab+Per was administered varied widely, from third-line to later-line settings, which may have influenced both efficacy and safety outcomes. Fourth, methods used to determine HER2 status were not uniform across institutions and included IHC, FISH, and NGS. This heterogeneity may have introduced variability in the identification of HER2-positive tumors and potentially influenced the study results. Fifth, OS may have been affected by subsequent therapies administered after disease progression. Because post-progression treatment were not standardized in this retrospective study, the impact of individual treatment strategies, including first-line biologic selection and anti-HER2 therapy, on OS should be interpreted with caution.

Conclusions

In clinical practice, first-line treatment regimens should be selected based on patient characteristics, including tumor biomarkers and the distinct toxicity profiles of available therapies. The Tmab+Per combination therapy was effective in real-world clinical settings; however, appropriate patient selection and careful management of IRRs are critical to optimize treatment outcomes.

Declarations

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Authors' Contributions

Shiho Kaneko and Kentaro Sawada conceived and designed the study. Shiho Kaneko developed the study protocol and obtained ethical approval. Shiho Kaneko collected and curated the data. Kentaro Sawada contributed to data collection and data curation. Kazuteru Hatanaka, Hiroshi Nakatsumi, Masayoshi Dazai, Takayuki Ando, Masahito Kotaka, Yasushi Tsuji, Michio Nakamura, Osamu Muto, Takashi Meguro, Takahiro Ishii, Atsushi Sato, and Susumu Sogabe collected data at their respective institutions. Shiho Kaneko and Kentaro Sawada performed the statistical analysis. Shiho Kaneko and

Kentaro Sawada drafted the manuscript. Shintaro Sawaguchi, Tatsuya Yokoyama, Koichi Ishida, Kazuaki Harada, Yasuyuki Kawamoto, Satoshi Yuki, Naoya Sakamoto, Yu Sakata, and Yoshito Komatsu critically revised the manuscript. Yoshito Komatsu supervised the study as the principal investigator and provided overall project oversight. All authors reviewed and approved the final manuscript.

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Data Availability

The datasets generated and/or analyzed during the current multicenter retrospective study are available from the corresponding author on reasonable request, subject to approval by the participating institutions.

Declarations:

Competing interests

Masahito Kotaka, Yasushi Tsuji, Satoshi Yuki, and Yoshito Komatsu have received honoraria from Chugai Pharmaceutical Co., Ltd. The remaining authors declare that they have no conflicts of interest.

Consent to participate

The requirement for obtaining informed consent from participants was waived owing to the retrospective nature of this study.

Ethical approval

The study was conducted in accordance with the principles outlined in the Declaration of Helsinki and was approved by the Ethics Committee of Hokkaido University Hospital (Clinical Research Number: IRB 023-0470).

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Tables

Table 1. Patient characteristics

		N=45 (%)
Age, year	Median (range)	63 (36-81)
Sex	Female	16 (35.6)
	Male	29 (64.4)
ECOG PS	0	30 (66.7)
	≥1	15 (33.3)
Primary tumor location	Left	33 (73.3)
	Right	12 (26.7)
Metastatic site	Liver	30 (66.7)
	Lung	16 (35.6)
	Lymph nodes	11 (24.4)
	Peritoneal dissemination	10 (22.2)
HER2 positivity diagnosed methods	IHC 3+	26 (57.8)
	FISH positive (except IHC3+)	15 (28.9)
	HER2 amp on NGS (except IHC 3+ or FISH positive)	4 (13.3)
RAS status	Wild	33 (73.3)
	Mutant	12 (26.7)
BRAF V600E status	Wild	45 (100)
	Mutant	0 (0)
MSI status	Non MSI-H	45 (100)
	MSI-H	0 (0)

Abbreviation: ECOG PS, Eastern Cooperative Oncology Group performance status; MSI-H, microsatellite instability-high; IHC, immunohistochemistry; FISH, fluorescence in situ hybridization; NGS, next-generation sequencing.

Table 2. Baseline characteristics of patients who received trastuzumab plus pertuzumab therapy

		N=20 (%)
Age, year	Median (range)	65 (36-80)
Sex	Female	9 (45.0)
	Male	11 (55.0)
Primary tumor location	Left	15 (75.0)
	Right	5 (25.0)
HER2 status	IHC 3+	9 (45.0)
	IHC 2+ and FISH +	7 (35.0)
	HER2 amplification on NGS or FISH	4 (20.0)
RAS status	Wild	15 (75.0)
	Mutant	5 (25.0)
Treatment lines	2nd	4 (20.0)
	3rd	1 (5.0)
	4th	7 (35.0)
	5th	5 (25.0)
	6th	3 (15.0)
RDI of Trastuzumab (%)	Median (range)	97.9 (68.6-100)
RDI of Pertuzumab (%)	Median (range)	97.7 (61.5-100)
Previous treatment	Anti-EGFR antibody	13 (65.0)
	Fluoropyrimidine	19 (95.0)
	Oxaliplatin	19 (95.0)
	Irinotecan	17 (85.0)
	Bevacizumab/Ramucirumab/Aflibercept	18 (90.0)
	Regorafenib	7 (35.0)
	Trifluridine/tipiracil	10 (50.0)
Premedication	Antihistamine ± NSAIDs	5 (25.0)
	No antihistamine ± NSAIDs	12 (60.0)

Abbreviations: FISH, fluorescence in situ hybridization; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; NGS, next-generation sequencing; NSAIDs, non-steroidal anti-inflammatory

drugs; RDI, relative dose intensity.

Table 3. AEs related Tmab+Per treatment

CTCAE v5.0, grade, N=20	1	2	3	4	Any grade	≥Grade 3
White blood cell decreased	1	1	0	0	2 (10.0)	0 (0)
Neutrophil count decreased	0	1	0	0	1 (5.0)	0 (0)
Anemia	3	2	1	0	6 (30.0)	1 (5.0)
Platelet count decreased	2	1	0	0	3 (15.0)	0 (0)
Nausea	2	0	0	0	2 (10.0)	0 (0)
Anorexia	3	3	0	0	6 (30.0)	0 (0)
Fatigue	5	3	0	0	8 (40.0)	0 (0)
Diarrhea	3	0	1	0	4 (20.0)	1 (5.0)
Fever	3	0	0	0	3 (15.0)	0 (0)
Infusion related reactions	3	2	0	1	6 (30.0)	1 (5.0)
Perimandibular inflammation	0	0	1	0	1 (5.0)	1 (5.0)
Cellulitis	0	0	1	0	1 (5.0)	1 (5.0)
Ureteral stones	0	0	1	0	1 (5.0)	1 (5.0)

Abbreviation: AEs, adverse events; CTCAE, Common Terminology Criteria for Adverse Events; Tmab, trastuzumab; Per, pertuzumab.

Table 4. Frequency of Infusion Related Reactions (IRRs) according to the premedication

Infusion Related Reactions	NSAIDs or Acetaminophen (+)	NSAIDs or Acetaminophen (-)	Total
Antihistamine (+)	1/6 (16.6%)	0/1 (0.0%)	1/7 (14.2%)
Antihistamine (-)	2/3 (66.7%)	3/9 (33.3%)	5/12 (41.7%)
Total	3/9 (33.3%)	3/10 (30.0%)	6/19 (31.6%)

Abbreviation: NSAIDs, nonsteroidal anti-inflammatory drugs.

Figures

Fig. 1 Study flow

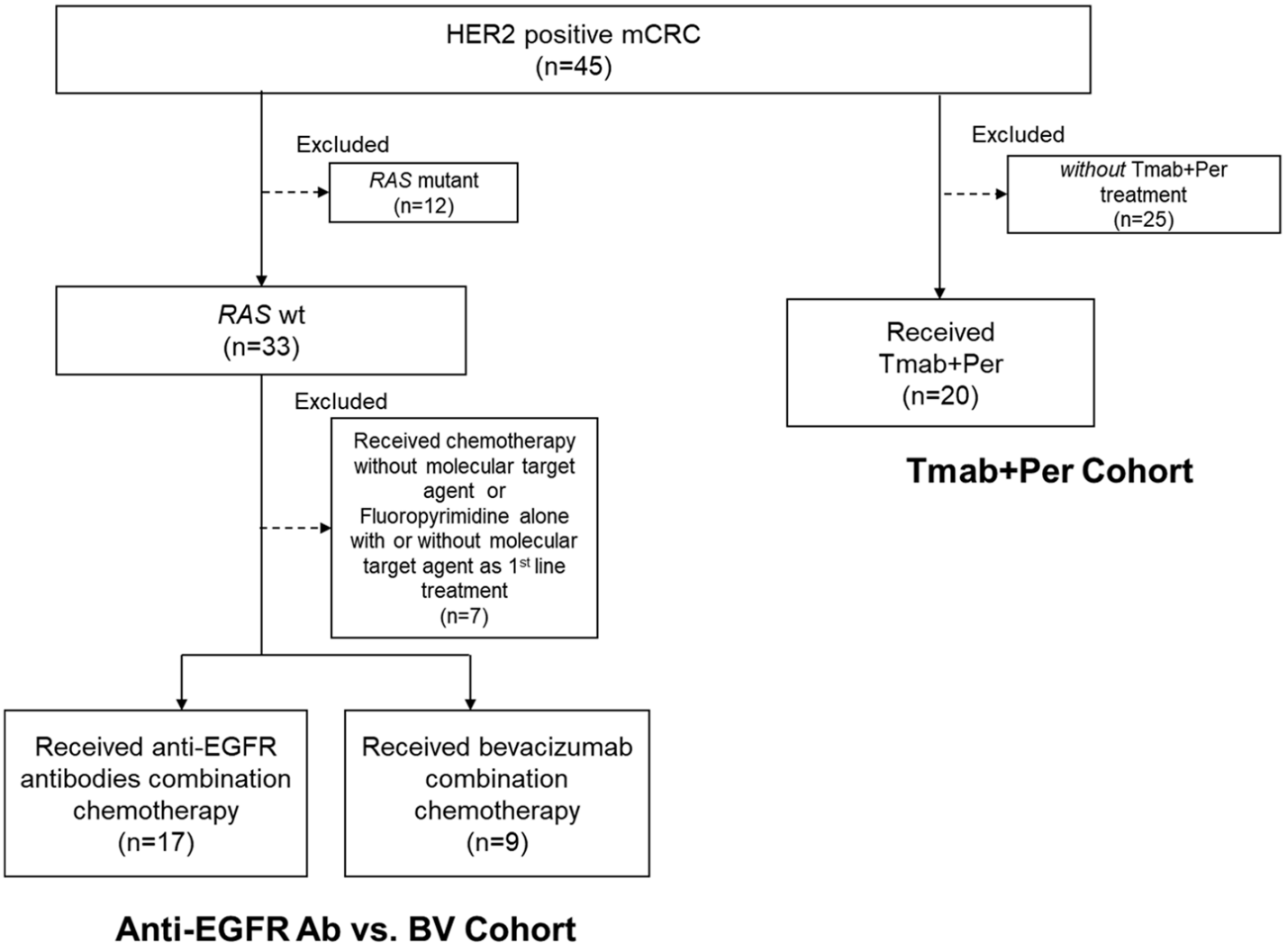


Figure 1

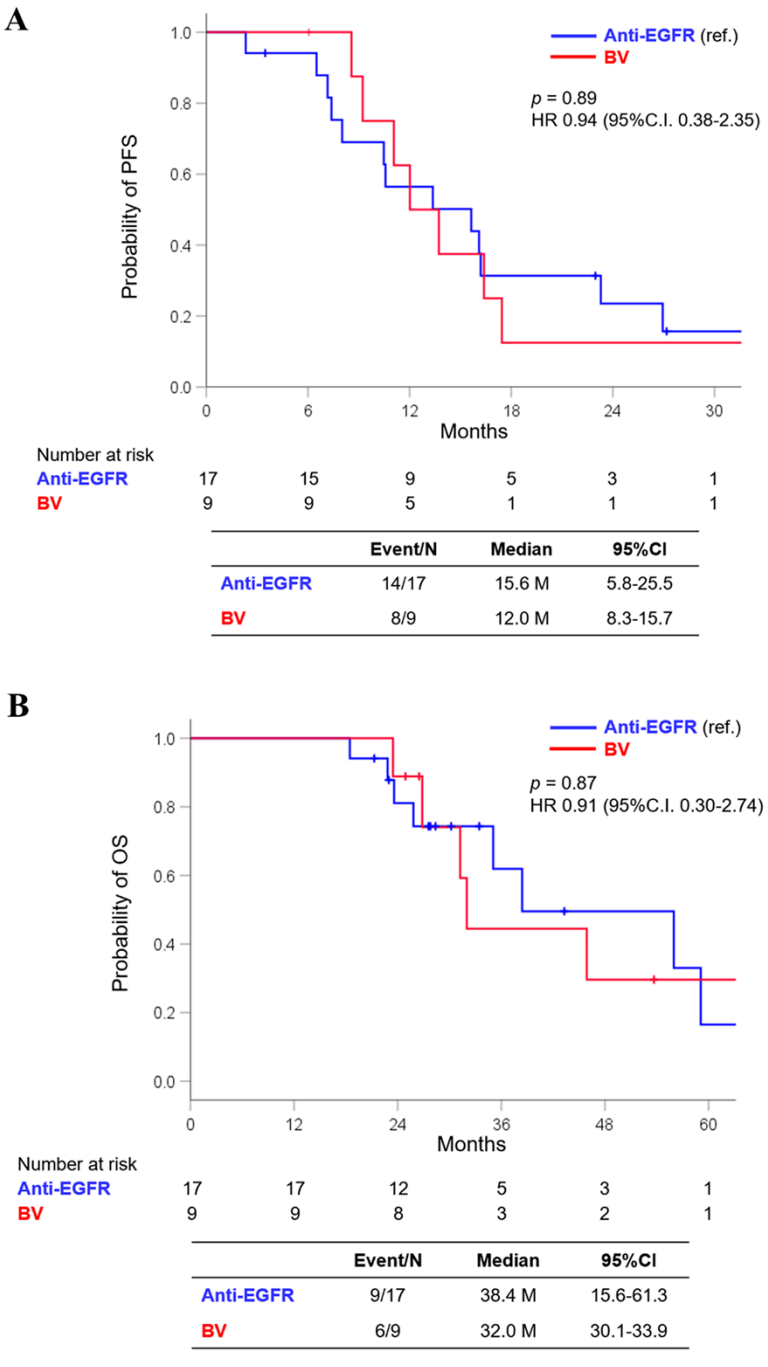
Study flow

A total of 45 patients with human epidermal growth factor receptor 2 (HER2)-positive metastatic colorectal cancer (mCRC) were identified. Among them, 12 patients had *RAS*-mutant tumors, and 33 had *RAS* wild-type (wt) tumors and were included in the analysis. Of the 33 *RAS* wt patients, seven who received fluoropyrimidine monotherapy (with or without molecular targeted agents) or chemotherapy without molecular targets as first-line therapy were excluded from the comparative analysis. The remaining patients were classified into the anti-epidermal growth factor receptor (EGFR) antibody plus chemotherapy group (*n* = 17) and the bevacizumab plus chemotherapy group (*n* = 9) in the anti-EGFR

antibody *vs*. bevacizumab (BV) cohort. In addition, 20 of the 45 patients received trastuzumab plus pertuzumab (Tmab+Per) and were included in the Tmab+Per cohort.

mCRC, metastatic colorectal cancer; wt, wild-type; Tmab+Per, trastuzumab plus pertuzumab; EGFR, epidermal growth factor receptor; BV, bevacizumab

Fig. 2 PFS (A) and OS (B) according to 1st line regimens in *RAS* wt patients



	Event/N	Median	95%CI
Anti-EGFR	14/17	15.6 M	5.8-25.5
BV	8/9	12.0 M	8.3-15.7

	Event/N	Median	95%CI
Anti-EGFR	9/17	38.4 M	15.6-61.3
BV	6/9	32.0 M	30.1-33.9

Figure 2

Progression-free survival (PFS) (A) and overall survival (OS) (B) according to first-line regimens in *RAS* wild-type (wt) patients

(A) Kaplan–Meier curves for PFS in *RAS* wt patients treated with anti-EGFR antibody plus chemotherapy or BV plus chemotherapy as first-line treatment.

(B) Kaplan–Meier curves for OS in *RAS* wt patients according to first-line therapy.

PFS, progression-free survival; OS, overall survival; wt, wild-type; EGFR, epidermal growth factor receptor; BV, bevacizumab

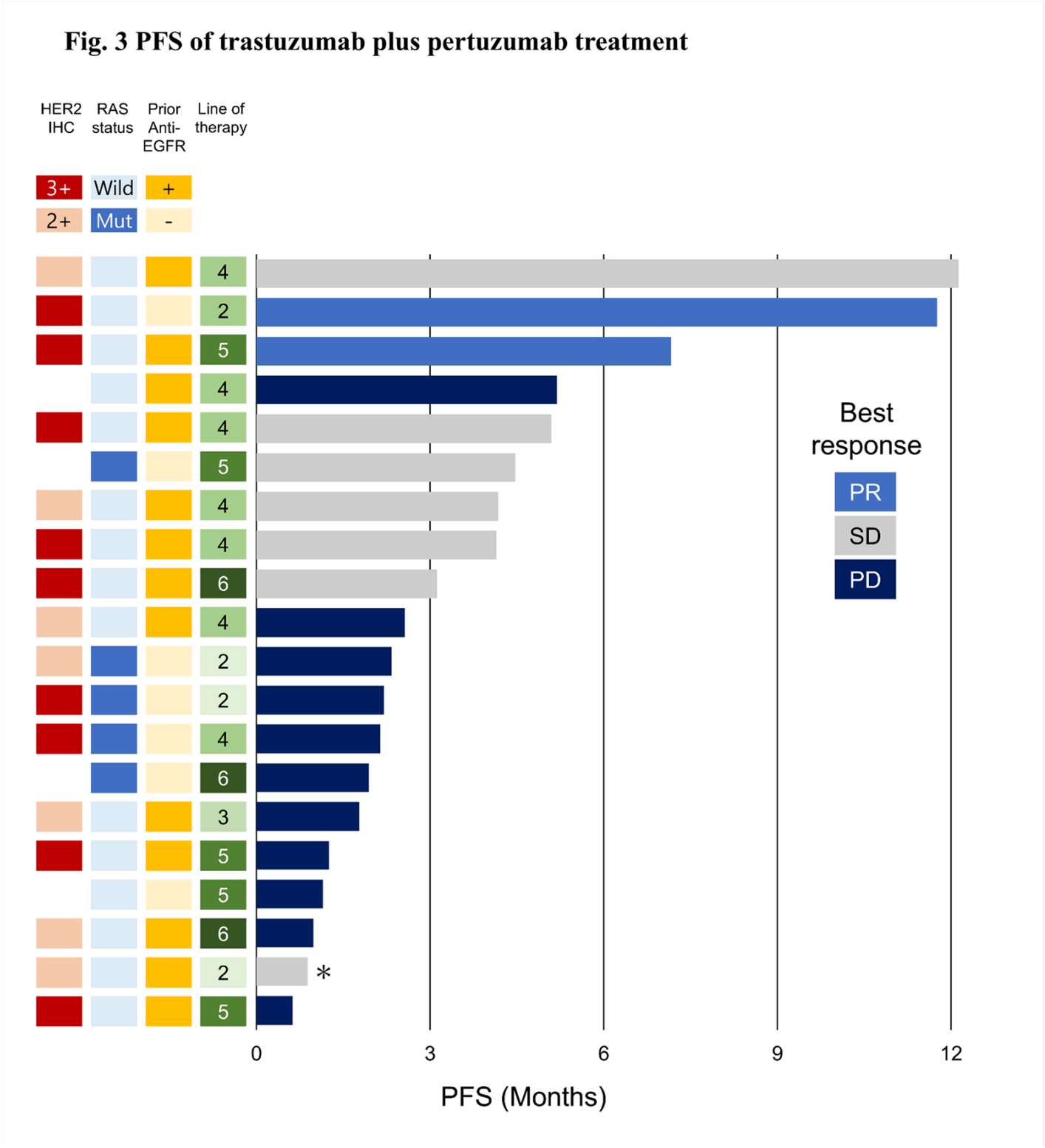


Figure 3

Progression-free survival (PFS) of trastuzumab plus pertuzumab (Tmab+Per) treatment

Swimmer plot showing PFS in individual patients who received Tmab+Per. Each horizontal bar represents the duration of PFS (months) for each patient. Colors indicate the best tumor response: partial response (PR), stable disease (SD), and progressive disease (PD). Baseline characteristics,

including HER2 immunohistochemistry (IHC) status, *RAS* status, prior anti-EGFR therapy, and line of therapy, are shown on the left. An asterisk (*) indicates a censored case due to treatment discontinuation caused by an adverse event. The remaining patients experienced progression events.

PFS, progression-free survival; PR, partial response; SD, stable disease; PD, progressive disease; IHC, immunohistochemistry; EGFR, epidermal growth factor receptor

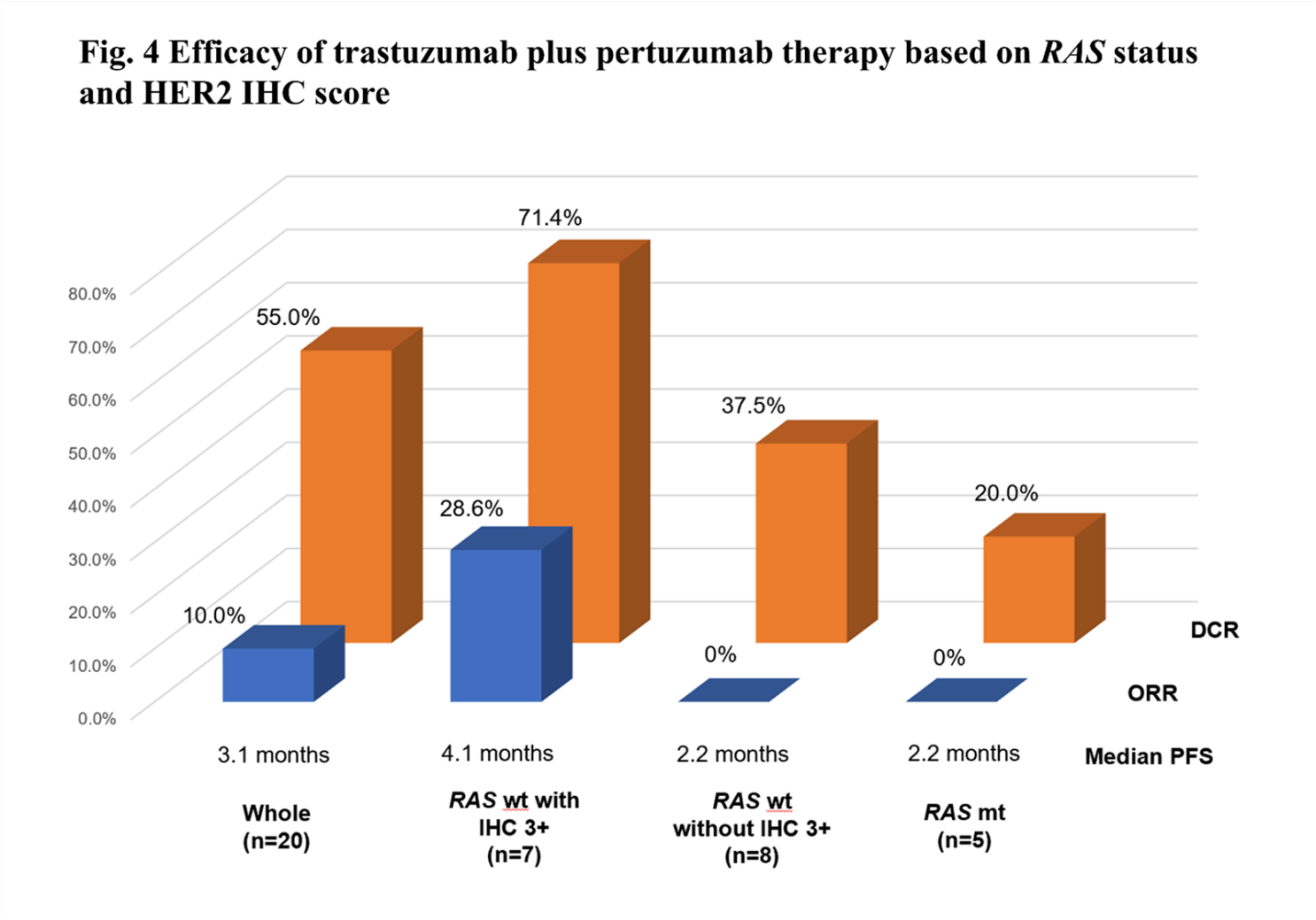


Figure 4

Efficacy of trastuzumab plus pertuzumab therapy based on *RAS* status and human epidermal growth factor receptor 2 (HER2) immunohistochemistry (IHC) score

Objective response rate (ORR) and disease control rate (DCR) are shown according to *RAS* mutation status and HER2 IHC score. ORR is indicated by blue bars, and DCR by orange bars.

ORR, objective response rate; DCR, disease control rate; IHC, immunohistochemistry

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- HGCSG2304suppletablever1.3.docx