Major comments #1.

As described in discussion part, this reviewer cannot agree with the latter part of the conclusion. Since the anti-HER2 therapy is now approved, the authors should compare treatment outcome of HER2 overexpression group with that of the non-overexpression group. Moreover, to demonstrate that HER2 overexpression is not prognostic factor for mCRC, the authors need to perform multivariate analysis for OS and demonstrate that HER2 overexpression is not an independent prognostic factor. If anti-HER2 therapy is not approved for mCRC in Korea, the authors should add "in Korea" after "These findings suggest that the status of HER2 expression need not be considered when choosing regimens as first- and second-line treatments."

Answer #1-1.

Thank you for your comment.
We agree on your opinion.

In HER2-positive patients with mCRC, treatment with HER2 dual blockade is optionally recommended. However, all of HER2 dual blockade treatment,such as trastuzumab-lapatinib (HERACLES trial), trastuzumab-pertuzumab (MYPATHWAY trial), and trastuzumab deruxtecan (DESTINY-CRC01 trial), have proven effective as third or more line. The standard of first and second line treatment for mCRC are the combination of anti-EGFR/anti-VEGF and 5-FU backbone chemotherapy regardless of HER-2 overexpression. We conducted an analysis on patients who received those standard treatments, thus also mentioned the limitation in the part of discussion.

Changes in the text #1-1:
We added sentences as follows;

In the part of 1.2 Rationale and knowledge gap,

"Moreover, all of the HER2 dual blockade treatment, trastuzumab-lapatinib (HERACLES trial), trastuzumab-pertuzumab (MYPATHWAY trial), and trastuzumab deruxtecan (DESTINY-CRC01 trial), have proven effective as third-line and beyond. The first- and second-line treatment standard for mCRC remains the combination of anti-EGFR/anti-VEGF and 5-FU backbone chemotherapy, regardless of HER-2 overexpression (28-31)."

"4.4 Limitations
This study had several limitations. First, this study had a small sample size and was retrospective in nature; our results should therefore be confirmed in a prospective study. Second, only Asian patients with mCRC were enrolled in the study, limiting the generalizability because of differences in the molecular profiles and clinical features between Western and Eastern patients with mCRC. Third, the analyses did not include patients who received anti-HER2-directed therapy."

We changed the conclusion according to your comment as flows;

"These findings suggest that the status of HER2 overexpression need not be considered when choosing regimens as current first- and second-line treatments."

Answer #1-2.
According to your comment, we conducted multivariate analysis for OS. In the univariate analysis conducted first, the variables that showed a significant meaning of overall survival with $P < 0.1$ was ECOG and BRAF mutation. In addition to these, clinically significant variables, such as age, gender, ECOG, location, and sidedness, were included in multivariate analysis.

Changes in the text #1-2:

We added the table 5 for this point as follows;

Table 5. Univariate Cox Regression Analyses of clinicopathological factors for the overall survival.

Variables	No. of patients	No. of Events	Univariate		Multivariate	
			HR (95% CI)	P	HR (95% CI)	P
Age	105	40	1.00 [0.98, 1.03]	0.758	1.00 [0.97, 1.02]	0.799
Sex			1.18 [0.61, 2.29]	0.620	0.95 [0.46, 1.97]	0.895
Male	65	26				
Female	40	14				
ECOG			4.27 [1.74, 10.5]	0.002*	6.05 [1.98, 18.5]	0.002*
0-1	96	34				
≥2	9	6				
Location			0.66 [0.33, 1.32]	0.238	0.55 [0.24, 1.25]	0.152
Colon	71	29				
Rectum	34	11				
Sidedness			0.78 [0.39, 1.56]	0.474	1.06 [0.46, 2.45]	0.884
Right	23	11				
Left	82	29				
Differentiation	(Total 83)					
Well	8	2	Reference			
Moderate	66	26	1.41 [0.33, 5.97]	0.640		
Poor	9	7	2.52 [0.50, 12.6]	0.260		
KRAS‡			1.09 [0.58, 2.07]	0.787		
wild type	59	23				
mutation	46	17				
NRAS‡			1.62 [0.22, 11.9]	0.634		
wild type	103	39				
mutation	2	1				
BRAF‡			2.70 [0.93, 7.80]	0.067	3.06 [1.03, 9.06]	0.043*
wild type	97	36				
mutation	8	4				
MMR status‡			1.68 [0.51, 5.55]	0.394	2.51 [0.59, 10.7]	0.212
MSS	101	37				
MSI-High	4	3				
TMB‡			1.02 [0.50, 2.09]	0.962		
Low	83	30				
High	22	10				
HER2			1.50 [0.57, 3.90]	0.411	1.56 [0.56, 4.34]	0.394
Negative	96	35				
Positive	9	5				

HR: Hazard ratio, BRAF: B-Raf Proto-Oncogene, ECOG: European Cooperative Oncology Group score, HER2: human epidermal growth factor receptor-2, KRAS: Kirsten ras oncogene homolog, MMR: mismatch repair, MSI: Microsatellite instability, MSS: microsatellite stable, NRAS: neuroblastoma RAS viral oncogene homolog, TMB: tumor mutational burden.
† Tumors located at the descending colon, sigmoid colon, and rectum were defined as left-sided colorectal cancer.
‡ KRAS, NRAS, and BRAF mutation, MMR status, and TMB were tested using next-generation sequencing. The cut-off value for TMB-high was 10 mutations/Megabase.

Major comment #2.

  The authors conducted multivariate analysis; however, the method of the analysis was not referred to. Additionally, the authors included a large number of variables compared to the number of events, although I understand that the incidence of mCRC patients with HER2 over expression is quite low. The authors should re-analyze using the appropriate method.

Answer #2.

Thank you for your comment.
We agree on your opinion and add the sentence for this point as follows in the part of method and the part of 3.3;

Changes in the text:

"Using Cox regression, we conducted univariate and multivariate analyses to assess prognostic factors. We estimated all univariate models and included independent variables with P<0.1 in the multivariate model. Also, clinically significant variables were included using background knowledge."

In the part of 3.3 Predictive and prognostic analysis according to HER2 expression and other variables,

"When HER2 overexpression was assessed as a prognostic marker, there was no evidence as an independent factor for PFS to first-line chemotherapy in multivariate analysis (hazard ratio [HR] , 0.82; 95% CI, 0.35–1.94, P =0.652). BRAF mutation and MSI-high were significant risk factors for the PFS to first-line treatment in univariate and multivariate analyses. The HR of BRAF mutation was 2.49 (95% CI 1.04–5.96, P=0.041), and the HR of MSI-high was 3.83 (95% CI 1.24–11.8, P=0.019), in multivariate analysis (Table 4). Similarly, there was no evidence that HER2 overexpression was an independent prognostic factor for OS in the multivariate analysis (HR, 1.50; 95% CI 0.57–3.90; P=0.411). ECOG and BRAF mutation were statistically significant, with HRs 6.05 (95% CI 1.98–18.5, P=0.002) and 3.06 (95% CI 1.03–9.06, P=0.043, Table 5), respectively."

Table 4 and Table 5 were revised.

Table 4. Univariate and Multivariate Cox Regression Analyses of clinicopathological factors for the progression-free survival to 1st line chemotherapy.

Variables	No. of patients	No. of Events	Univariate HR (95% CI)	P	Multivariate HR (95% CI)	P
Age	105	79	0.99 [0.97, 1.01]	0.195	0.99 [0.97, 1.01]	0.407
Sex			1.20 [0.76, 1.90]	0.438	1.20 [0.73, 1.96]	0.480
Male	65	49				
Female	40	30				
ECOG			1.32 [0.61, 2.89]	0.481	1.57 [0.66, 3.76]	0.311
0-1	96	72				
≥2	9	7				
Location			1.08 [0.67, 1.75]	0.739	0.99 [0.58, 1.69]	0.965
Colon	71	54				
Rectum	34	25				
Sidedness						
Right	23	15	1.08 [0.61, 1.90]	0.793	1.06 [0.54, 2.07]	0.860
Left†	82	64				
Differentiation						
Well	8	4	Reference			
Moderate	66	53	0.96 [0.35, 2.69]	0.944		
Poor	9	9	0.76 [0.22, 2.60]	0.658		
KRAS‡			1.03 [0.65, 1.64]	0.892		

Variables	No. of patients	No. of Events	Univariate HR (95% CI)	P	Multivariate HR (95% CI)	P
wild type	59	46				
mutation	46	33				
NRAS‡			0.75 [0.18, 3.11]	0.690		
wild type	103	77				
mutation	2	2				
BRAF‡			2.53 [1.07, 6.00]	0.034*	2.49 [1.04, 5.96]	0.041*
wild type	97	73				
mutation	8	6				
MMR status‡			4.06 [1.44, 11.5]	0.008*	3.83 [1.24, 11.8]	0.019*
MSS	101	75				
MSI-High	4	4				
TMB‡			1.13 [0.67, 1.92]	0.648		
Low	83	60				
High	22	19				
HER2			0.73 [0.33, 1.61]	0.433	0.82 [0.35, 1.94]	0.652
Negative	96	72				
Positive	9	7				

HR: Hazard ratio, BRAF: B-Raf Proto-Oncogene, ECOG: European Cooperative Oncology Group score, HER2: human epidermal growth factor receptor-2, KRAS: Kirsten ras oncogene homolog, MMR: mismatch repair, MSI: Microsatellite instability, MSS: microsatellite stable, NRAS: neuroblastoma RAS viral oncogene homolog, TMB: tumor mutational burden.
† Tumors located at the descending colon, sigmoid colon, and rectum were defined as left-sided colorectal cancer.
‡ KRAS, NRAS, and BRAF mutation, MMR status, and TMB were tested using next-generation sequencing. The cut-off value for TMB-high was 10 mutations/Megabase.

Table 5. Univariate Cox Regression Analyses of clinicopathological factors for the overall survival.

Variables	No. of patients	No. of Events	Univariate HR (95% CI)	P	Multivariate HR (95% CI)	P
Age	105	40	1.00 [0.98, 1.03]	0.758	1.00 [0.97, 1.02]	0.799
Sex			1.18 [0.61, 2.29]	0.620	0.95 [0.46, 1.97]	0.895
Male	65	26				
Female	40	14				
ECOG			4.27 [1.74, 10.5]	0.002*	6.05 [1.98, 18.5]	0.002*
0-1	96	34				
≥2	9	6				
Location			0.66 [0.33, 1.32]	0.238	0.55 [0.24, 1.25]	0.152
Colon	71	29				
Rectum	34	11				
Sidedness			0.78 [0.39, 1.56]	0.474	1.06 [0.46, 2.45]	0.884
Right	23	11				
Left	82	29				
Differentiation	(Total 83)					
Well	8	2	Reference			
Moderate	66	26	1.41 [0.33, 5.97]	0.640		
Poor	9	7	2.52 [0.50, 12.6]	0.260		
KRAS‡			1.09 [0.58, 2.07]	0.787		
wild type	59	23				
mutation	46	17				
NRAS‡			1.62 [0.22, 11.9]	0.634		
wild type	103	39				
mutation	2	1				

			HR [95% CI]	p	HR [95% CI]	p
BRAF[‡]			2.70 [0.93, 7.80]	0.067	3.06 [1.03, 9.06]	0.043*
wild type	97	36				
mutation	8	4				
MMR status[‡]			1.68 [0.51, 5.55]	0.394	2.51 [0.59, 10.7]	0.212
MSS	101	37				
MSI-High	4	3				
TMB[‡]			1.02 [0.50, 2.09]	0.962		
Low	83	30				
High	22	10				
HER2			1.50 [0.57, 3.90]	0.411	1.56 [0.56, 4.34]	0.394
Negative	96	35				
Positive	9	5				

HR: Hazard ratio, BRAF: B-Raf Proto-Oncogene, ECOG: European Cooperative Oncology Group score, HER2: human epidermal growth factor receptor-2, KRAS: Kirsten ras oncogene homolog, MMR: mismatch repair, MSI: Microsatellite instability, MSS: microsatellite stable, NRAS: neuroblastoma RAS viral oncogene homolog, TMB: tumor mutational burden.
[†] Tumors located at the descending colon, sigmoid colon, and rectum were defined as left-sided colorectal cancer.
[‡] KRAS, NRAS, and BRAF mutation, MMR status, and TMB were tested using next-generation sequencing. The cut-off value for TMB-high was 10 mutations/Megabase.

Major comment #3.

This study evaluated the treatment outcome of mCRC patients for whom chemotherapy data were available; therefore, the authors should provide a summary of patients' background for "evaluable patients."

Answer #3.

Thank you for your comment.
As you advised, we added the data in Table1 as follows;

Changes in the text:

We revised the Table 1 as below.

First-line chemotherapy regimen			
Bevacizumab + FOLFIRI	27 (25.7)	25 (26.0)	2 (20.0)
Bevacizumab + FOLFOX	63 (60.0)	57 (59.4)	6 (60.0)
Cetuximab + FOLFIRI	9 (8.6)	8 (8.3)	1 (0.0)
Cetuximab + FOLFOX	0 (0.0)	0 (0.0)	0 (0.0)
Other[‡]	6 (5.4)	6 (6.3)	0 (0.0)
Second-line chemotherapy regimen	(n = 64)		
Bevacizumab + FOLFIRI	28 (43.6)	24 (41.4)	4 (66.7)
Bevacizumab + FOLFOX	19 (29.9)	17 (29.3)	2 (33.3)
Cetuximab + FOLFIRI	1 (1.6)	1 (1.7)	0 (0.0)
Cetuximab + FOLFOX	0 (0.0)	0 (0.0)	0 (0.0)
Aflibercept + FOLFIRI	10 (15.6)	10 (17.2)	0 (0.0)
Aflibercept + FOLFOX	1 (1.6)	1 (1.7)	0 (0.0)
Other$	5 (7.8)	5 (8.6)	0 (0.0)

Major comment #4.

The number at risk in Figures 2 and 3 appears to be wrong. The number at risk should always start with the population size. Moreover, PFS of HER2 overexpression group tends to be better than HER2 non-overexpression group, whereas OS of HER2 overexpression group tends be worse. Please confirm this and re-analyze the data.

Answer #4.

Thank you for your comment.
We agree on your opinion.

We rechecked the figures and corrected the number at risk in Figure 2 and 3.
We added sentences for this point as follows;

Changes in the text:

In the part of 3.3 Predictive and prognostic analysis according to HER2 expression and other variables,

"Among patients who underwent first-line chemotherapy, the median PFS was 11.05 months (95% CI 9.416–12.682), and there was no difference in PFS to first-line chemotherapy between HER2-positive and -negative tumors (P=0.431, Figure 2A). The median PFS to second-line chemotherapy was also not different between HER2-positive and -negative tumors (P= 0.861; Figure 2B). The median OS of the 105 patients was 37.74 months (95% CI 26.562–49.914). Like PFS, the median OS was longer in the HER2-positive group, but this lacked statistical significance (P=0.245, Figure 3)."
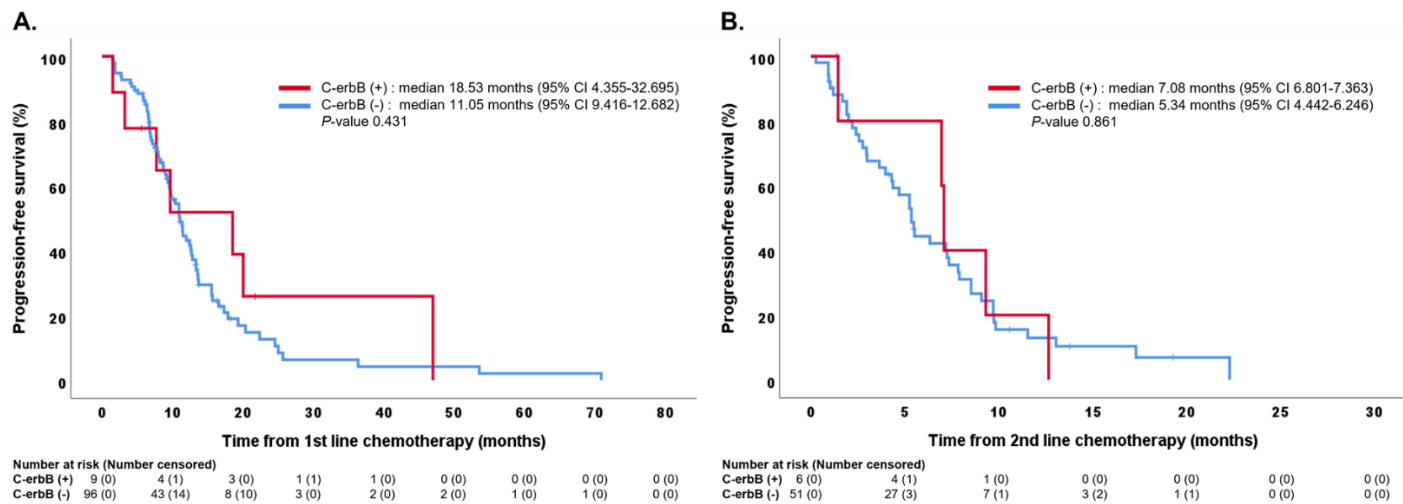

A.

Progression-free survival (%)

C-erbB (+) : median 18.53 months (95% CI 4.355-32.695)
C-erbB (-) : median 11.05 months (95% CI 9.416-12.682)
P-value 0.431

Time from 1st line chemotherapy (months)

Number at risk (Number censored)

C-erbB (+)	9 (0)	4 (1)	3 (0)	1 (1)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)
C-erbB (-)	96 (0)	43 (14)	8 (10)	3 (0)	2 (0)	2 (0)	1 (0)	1 (0)	0 (0)

B.

Progression-free survival (%)

C-erbB (+) : median 7.08 months (95% CI 6.801-7.363)
C-erbB (-) : median 5.34 months (95% CI 4.442-6.246)
P-value 0.861

Time from 2nd line chemotherapy (months)

Number at risk (Number censored)

C-erbB (+)	6 (0)	4 (1)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)
C-erbB (-)	51 (0)	27 (3)	7 (1)	3 (2)	1 (1)	0 (0)	0 (0)

Figure 2. Kaplan-Meier estimates of progression-free survival. HER2: human epidermal growth factor receptor-2, CI: Confident interval, PFS: Progression-free survival, mCRC: metastatic colorectal cancer. The median follow-up duration was 22.6 months (range, 1.93-92.17). There was no statistically significant difference in PFS for first- and second-line chemotherapy, respectively, between HER2-positive and HER2-negative mCRC. **(A)** PFS for first-line chemotherapy. Median PFS was 18.53 months (95% CI 4.355-32.695) for HER2-positive and 11.05 months (95% CI 9.416-12.682, P=0.431) for HER2-negative mCRC. **(B)** PFS for second-line chemotherapy. Median PFS was 7.08 months (95% CI 6.801-7.363) for HER2-positive and 5.34 months (95% CI 4.442-6.246, P=0.861) for HER2-negative mCRC.
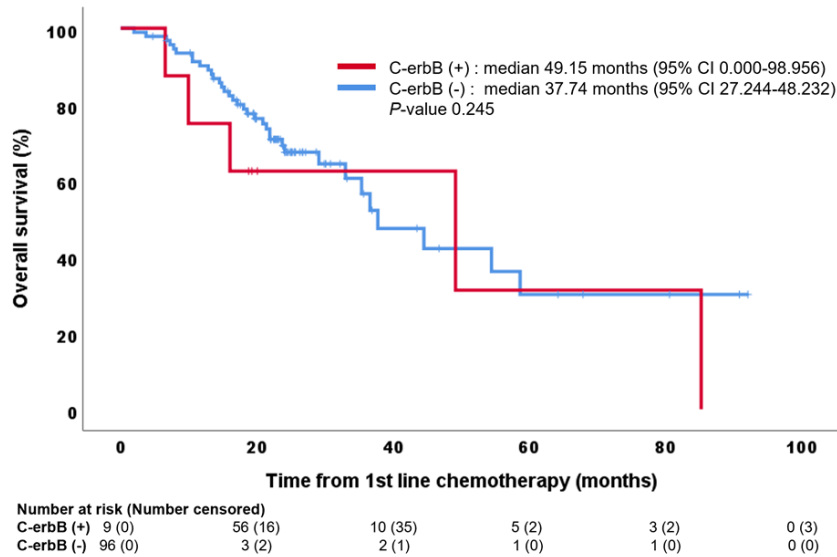
Figure 3. Kaplan-Meier estimates of overall survival. HER2: human epidermal growth factor receptor-2, CI: Confident interval, mCRC: metastatic colorectal cancer. There was no difference in overall survival between HER2-positive and HER2-negative mCRC. Median overall survival was 49.15 months (95% CI 0.000-98.956) vs. 37.74 months (95% CI 27.244-48.232, *P*=0.245).

Minor comments

#1 The incidence of gastric cancer with HER2 overexpression is reported to be about 20%. The authors need to reconfirm the reference (Line 70).

Answer #1.

Thank you for your comment.
As you advised, we reconfirmed the reference as follows;.

Changes in the text:

In the part of 1.1 Background, Line 70-71,

"Dysregulated overexpression or amplification of HER2 drives oncogenesis (13), occurring in about 20% of invasive breast cancer (14), about 20% of gastric cancer(15), and about 1−30% of lung (16)."

#2 There are two different IRB numbers (IRB No. 2022-12-067 and IRB No. 2022-12-0670). Which number is correct? Please confirm.

Answer #2.

Thank you for your comment.
We corrected IRB numbers as follows;

Changes in the text:

In the part of Footnote, Line 265,
"IRB No. 2022-12-067"

#3 The authors used HERACLESS criteria to define HER2 overexpression, which include IHC2+/ISH+ as HER2 overexpression. However, the authors did not include IHC2+/ISH+ and should provide a reason for this omission.

Answer #3.

Thank you for your comment.
We agree on your opinion we added the sentence for this point as follows;

Changes in the text:

In the part of 2.2 HER-2 (c-erbB-2) immunohistochemistry test, Line 114-118
"HER2 positive tumor was defined as IHC intensity score 3+ in more than 50% of the tumor cells, IHC intensity score 3+ in 10-50% of the tumor cell and fluorescence in situ hybridization (FISH) positive, or IHC intensity score 2+ in more than 50% of the tumor cells and FISH positive. FISH positive was defined as a HER2:CEP17 ratio is higher than two in more than 50% of the tumor cells."

#4 "the Response Criteria Evaluation in Solid Tumors (RECIST)" is likely a typographical error and should be corrected to "the Response Evaluation Criteria in Solid Tumors (RECIST)." (Line 113)

Answer #4.

Thank you for your comment.
We agree on your opinion and corrected it as follows;

Changes in the text:

In the part of 2.3. Outcomes and statistical analyses, Line 121-122
"Response Evaluation Criteria in Solid Tumors (RECIST)"

#5 "HER" appears to be a typographical error and should be corrected to "HER2" (Line 127).

Answer #5.

Thank you for your comment.
We corrected it as follows; .

Changes in the text:

In the part of 3.1. Patient characteristics, Line 138
"HER2 IHC test"

#6 The authors should insert "Table2" and "Table3" in the section discussing the Efficacy of chemotherapy according to HER2 expression part (3.2).

Answer #6.

Thank you for your comment.
We insert "Table 2" and "Table 3"as follows;

In the part of 3.2. Efficacy of chemotherapy according to HER2 expression, Line 157 and 164,

"For patients who underwent first-line chemotherapy, as shown in Table 2," and "Concerning second-line chemotherapy, there were no significant differences in ORR (P=1.000) and DCR (P=1.000) in mCRC patients with HER2-positive and

negative tumors (Table3)."

#7 There is no Figure3-B. If the authors did not intent to analyze the OS of second-line treatment, they need to correct "Figure3-A" to "Figure3."

Answer #7.

Thank you for your comment.
We corrected "Figure3-A" to "Figure 3"as follows;

Changes in the text:

In the part of 3.3 Predictive and prognostic analysis according to HER2 expression and other variables., Line 173,
"but there was no statistical significance (P=0.245, Figure 3)."

#8 The authors should mention HER2 status in patients initially excluded 6 patients who were missing data in Figure1. Additionally, the authors need to remove PFS from the footnote in Figure2 and OS from Figure3.

Answer #8.

Thank you for your comment.
We agree on your opinion and we corrected it as follows;

Changes in the text:

In the part of 3.1. Patient characteristics, Line 139-141
"Among them, 6 patients were excluded for lack of data on chemotherapies and tumor response; 1 was HER2-positive, and 5 were HER2-negative."

In the footnote of Figure 2, the abbreviation "OS" was removed.
In the footnote of Figure 3, the abbreviation "PFS" was removed.