Manuscript **"Does *KRAS* codon 13 mutation have prognostic value in colorectal cancer?"**

Dear Editors of the World Journal of Gastroenterology,

We deeply thank the editorial staff and reviewers of the *World Journal of Gastroenterology* for reviewing our manuscript entitled **"Does *KRAS* codon 13 mutation have prognostic value in colorectal cancer?"** (Manuscript ID 85211).

We have substantially revised our manuscript in accordance with the reviewers' comments. The changes made in response to the individual comments have been described in the following pages. This paper was written in accordance with the guidelines of the *World Journal of Gastroenterology*. This manuscript has not been published before, nor is it under consideration for publication elsewhere. We hope that the changes made to our manuscript satisfy the reviewers' comments and meet the requirements for publication in the *World Journal of Gastroenterology*.

We wish to thank you again for the constructive comments provided by the reviewers. We hope that this article will be of interest to the readers of the *World Journal of Gastroenterology*.

Yours sincerely,

On behalf of the authors of this manuscript,

Duck-Woo Kim, M.D., Ph.D.

Professor, Department of Surgery, Seoul National University Bundang Hospital,

Korean Hereditary Tumor Registry, Cancer Research Institute, Seoul National University College of Medicine, 166 Gumi-ro, Bundang-gu, 463-707 Seongnam, Korea

Phone: +82-31-787-7101 Fax: +82-31-787-4055 E-mail: kdw@snubh.org

RESPONSES TO THE REVIEWERS' COMMENTS

Reviewer #1:

About 40% of colorectal cancer patients are associated with the mutation of the oncogene KRAS. In this study, the clinical and pathological characteristics of the single codon 12, 13 and 61 of the mutant KRAS gene in 2203 clinical cases of colorectal cancer from I to III were statistically analyzed. The results showed that KRAS codon 12 mutation was significantly associated with the pathological features closely related to tumor recurrence. Unlike codon 12, KRAS codon 13 mutation had little effect on the pathological features and recurrence. With large sample size, proper research methods and clear logic, this study provides theoretical basis for prognostic biomarkers of colorectal cancer patients, and has certain clinical significance.

However, in view of the shortcomings of this study, the following questions are suggested:

1. The title of most SCI papers uses declarative sentences to summarize and represent the main research content of the paper, especially research papers. For papers with definite conclusions obtained through experimental research, it is suggested that the title should not be used as a question, but directly use a clear and explicit statement as the title, which can more accurately reflect the research content of the paper.

- ***Response:*** Thank you for the comment on changing the title of our study to reflect the research content of the paper. The conclusion of our study was that the *KRAS* codon 13 mutation is less likely to serve as a prognostic factor for colorectal cancer compared with the *KRAS* codon 12 mutation. Therefore, we changed the title to cover the details of our study results. Notably, there are different oncologic features of the codon-specific *KRAS* mutation in colorectal cancer.

- *<Correction> Altered title:*

  Different oncological features of colorectal cancer codon-specific *KRAS* mutations: Not codon 13 but codon 12 have prognostic value

2. Abstract is an important part for readers to understand the research accurately and quickly, and also make the article fuller. The background of this research abstract is too short, so it is suggested to supplement it.

- ***Response:*** We thank the reviewer for the constructive comment regarding the abstract. Thank you for your review and we totally agree with your comments on the importance of the abstract and that it should contain the whole contents of the article. We have amended and supplemented the BACKGROUND part of the abstract to with the whole contents of the Introduction in our manuscript.

- *<Correction> Amended paragraph: Abstract, BACKGROUND*

  Approximately 40% of colorectal cancer (CRC) cases are linked to Kirsten rat sarcoma viral oncogene homolog (*KRAS)* mutations. *KRAS* mutations are associated with poor CRC prognosis, especially *KRAS* codon 12 mutation, which is associated with metastasis and poorer survival. However, the clinicopathological characteristics and prognosis of *KRAS* codon 13 mutation in CRC remain unclear.

3. The proportion of relevant references in the past three to five years is too small, so it is recommended to cite newer references.

- ***Response:*** Thank you for your thorough review. We agree that some of our cited references are old studies and have revised and added up-to-date references as follows.

- *<Correction> Added references*

3 Wan XB, Wang AQ, Cao J, Dong ZC, Li N, Yang S, Sun MM, Li Z, Luo SX. Relationships among KRAS mutation status, expression of RAS pathway signaling molecules, and clinicopathological features and prognosis of patients with colorectal cancer. World J Gastroenterol 2019; 25(7): 808-823 [PMID: 30809081 PMCID: PMC6385012 DOI: 10.3748/wjg.v25.i7.808]

7 Fan JZ, Wang GF, Cheng XB, Dong ZH, Chen X, Deng YJ, Song X. Relationship between mismatch repair protein, RAS, BRAF, PIK3CA gene expression and clinicopathological characteristics in elderly colorectal cancer patients. World J Clin Cases 2021; 9(11): 2458-2468 [PMID: 33889611 PMCID: PMC8040173 DOI: 10.12998/wjcc.v9.i11.2458]

11 Asawa P, Bakalov V, Kancharla P, Abel S, Chahine Z, Monga DK, Kirichenko AV, Wegner RE. The prognostic value of KRAS mutation in locally advanced rectal cancer. Int J Colorectal Dis 2022; 37(5): 1199-1207 [PMID: 35484252 DOI: 10.1007/s00384-022-04167-x]

39 Tonello M, Baratti D, Sammartino P, Di Giorgio A, Robella M, Sassaroli C, Framarini M, Valle M, Macri A, Graziosi L, Coccolini F, Lippolis PV, Gelmini R, Deraco M, Biacchi D, Santullo F, Vaira M, Di Lauro K, D'Acapito F, Carboni F, Giuffre G, Donini A, Fugazzola P, Faviana P, Sorrentino L, Scapinello A, Del Bianco P, Sommariva A. Microsatellite and RAS/RAF Mutational Status as Prognostic Factors in Colorectal Peritoneal Metastases Treated with Cytoreductive Surgery and Hyperthermic Intraperitoneal Chemotherapy (HIPEC). Ann Surg Oncol 2022; 29(6): 3405-3417 [PMID: 34783946 DOI: 10.1245/s10434-021-11045-3]

40 Formica V, Sera F, Cremolini C, Riondino S, Morelli C, Arkenau HT, Roselli M. KRAS and BRAF Mutations in Stage II and III Colon Cancer: A Systematic Review and Meta-Analysis. J Natl Cancer Inst 2022; 114(4): 517-527 [PMID: 34542636 PMCID: PMC9002292 DOI: 10.1093/jnci/djab190]

4. In case statistics, the reasons for excluding patients with stage IV colorectal cancer are suggested in the front instead of in the discussion section.

- ***Response:*** Thank you for your insightful review. We agree that the reasons for excluding patients with stage IV colorectal cancer should be in the forefront in the MATERIALS AND METHODS section. Thus, we revised the paragraph describing the patients to understand why we had to exclude stage IV metastatic colorectal cancer patients. In addition, since understanding the prognostic value of each codon of the *KRAS* mutation is too complex in stage IV colorectal cancer, we think it is worth separately discussing with the readers of our paper through the DISCUSSION section, instead of setting it aside as one of the limitations of the study. Therefore, we revised the contents of stage IV issues into a separate paragraph and repositioned them before the "limitations" paragraph.

- *<Correction #1> Revised paragraph: MATERIALS AND METHODS - Patients*

  This retrospective observational cohort study was registered at ClinicalTrials. gov (NCT05657210) and reviewed 3,144 patients who underwent surgery for CRC between January 2009 and December 2019, with available clinical data on recurrence and survival. All patients underwent routine colon or rectal resection and lymph node dissection according to the tumor location, with or without diverting ileostomies or colostomies. The surgical specimens were submitted to the laboratory for pathological evaluation. Patients with confirmed molecular pathology reports of *KRAS* mutation status were included, whereas those with incomplete data on *KRAS* mutations (n=368) or microsatellite instability (MSI) status (n=232) were excluded. Patients with dual or triple *KRAS* mutations (within more than one codon) from pathology reports (n=2) were excluded. Additionally, to understand the biological importance and minimize the potential influence of systemic therapeutic factors on the prognosis of codon-specific *KRAS* mutations, we excluded patients with stage IV

metastatic CRC (n=339). Finally, data from 2,203 eligible patients were collected separately for statistical analysis. This study was approved by the Institutional Review Board (IRB No. B-2203-742-101) of Seoul National University Bundang Hospital and the requirement for informed consent was waived.

- *<Correction #2> Repositioned and revised paragraph: DISCUSSION*

- Clarifying the effects of codon-specific *KRAS* mutations on the prognosis of stage IV CRC is a complex issue. A recent study on *KRAS* mutations in CRC with liver metastasis reported that *KRAS* codon 12 mutations were associated with poorer overall survival, while codon 13 was not; however, they also pointed out the exclusion of perioperative management such as anti-epidermal growth factor receptor agents[9]. Among the patients diagnosed with stage IV CRC who underwent surgery in our hospital during the period of the present study, 48.4% had *KRAS* mutations. However, only about half of them (53.1%) underwent surgery with curative intent, whereas the others underwent palliative treatment. Additionally, there is a wide range of variations in the metastatic burden and forms of treatment for these patients. Therefore, in the present study, we excluded stage IV disease to focus on the biological importance and prognostic impact of codon-specific *KRAS* mutations in stage I–III CRC.

5. The data collection part of materials method, KRAS mutation result diagram and MSI state result diagram analysis manuscript are missing.

- ***Response:*** Thank you for your comments. The analysis of the frequency of *KRAS* mutation and MSI status was included in the descriptive statistics for the baseline characteristics, which we have mentioned in the "MATERIALS AND METHODS, *Statistical analysis*" section. The reason to draw the diagram for *KRAS* mutation and MSI status is to visualize and to make it easier for readers to understand at a glance. To avoid confusion, we have amended the sentence that explains the statistical methods for identifying the basic characteristics of

patients.

- *<Correction> Amended paragraph: MATERIALS AND METHODS - Statistical analysis*

Descriptive statistics were used to identify the basic clinicopathological characteristics of the patients, including MSI status frequency and *KRAS* mutations. The differences between wild-type and mutant *KRAS* as well as the mean values of continuous variables, were compared using either the independent t-test or the Mann–Whitney U test according to the results of the Kolmogorov-Smirnov test. Chi-squared or Fisher's exact tests were used to compare categorical variables. Overall survival (OS) and recurrence-free survival (RFS) were calculated from the date of surgery and compared using the Kaplan–Meier method and the log-rank test. For the analysis of risk factors for tumor recurrence, the Cox proportional hazards regression model was used, with the covariance input criterion set at $P < 0.1$. Patients were subdivided based on the primary tumor location (colon versus rectum) and MSI status (microsatellite stable (MSS)/MSI-low versus MSI-high). Each subgroup was analyzed for recurrence-related factors using a Cox proportional hazards regression model. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), version 25.0, for Windows (SPSS, IBM). Descriptive results of continuous variables are expressed as mean ± standard deviation. P value < 0.05 were considered statistically significant.

Reviewer #2:

This article examines the relationship between KRAS mutations in colorectal cancer (CRC) and patient prognosis. A large cohort was used and KRAS codon 12 mutations were found to be associated with a poorer prognosis, while codon 13 mutations were not significantly associated with pathological features or recurrence. However, the study has a number of limitations, including selectivity bias and missing data, which need to be noted. Weaknesses: The study did

not consider the impact of BRAF mutations on CRC prognosis, which is a key biomarker. The study was a single-centre, retrospective study with selectivity bias. Since KRAS mutations were assessed using postoperative specimens, there may be bias.

- ***Response:*** Thank you for the reviews and we strongly agree that this study has inevitable limitations and bias that could weaken our conclusion. The selective bias was due to the retrospective study design using postoperative specimens and missing data from patients who could not be contacted. Despite constant updates by the assigned research nurses, refusal to revisit after a few follow-ups could have resulted in the missing data of our cohort. The impact of the *BRAF* mutation was omitted, even though it considers a poor prognostic factor on colorectal cancer. The protocol of routine molecular examinations was altered within our study period. Additionally, nowadays only stage IV colorectal cancer patients undergo the *BRAF* mutation test from the cancerous samples in our hospital to determine the possibility of immunotherapy.

  Despite these limitations, the strength of our study lies in being a large-scale cohort with a relatively well-organized colorectal cancer registry of patients who underwent surgery. Additionally, this cohort is the largest ever to analyze the codon-specific *KRAS* mutation. Therefore, our results could not only support the previous studies, but also propose that the *KRAS* codon 13 mutation is less likely to serve as a prognostic factor for colorectal cancer.

Reviewer #3:

Thanks a lot for submission of fully detailed cohort study. All text of the manuscript need to be revised by a native English language editor.

- ***Response:*** Thank you for your insightful review of our work. As advised, we will be proceeding with English editing for the final revised manuscript.

The comments are indicated in the body of manuscript. So, the changes may be shown track-changed and/or Highlighted.

- ***Response:*** Thank you very much for the thorough review and comments. Some small changes, such as correcting tense errors, re-phrasing of sentences, and redesigning of the table were amended as you have advised. Since the final revised manuscript will be edited by a native English editor, it may not be able to show the tracked changes. We did, however, highlighted the changes in the final manuscript after the English editing. The following comments below, which you have commented on the manuscript, were listed with individual responses;

1) You may change the title to cover full/more details of the study

- ***Response:*** Thank you for the comment on changing the title of our study to reflect the research content of the paper. There are different oncologic features of the codon-specific *KRAS* mutation in colorectal cancer. The conclusion of our study was that the *KRAS* codon 13 mutation is less likely to serve as a prognostic factor for colorectal cancer compared with the *KRAS* codon 12 mutation. Therefore, we changed the title to cover the details of our study results.

- *<Correction> Altered title:*

  Different oncological features of colorectal cancer codon-specific *KRAS* mutations: Not codon 13 but codon 12 have prognostic value

2) Please kindly specify the Authors' Contribution with details and explore the role of

authors in this study.

- ***Response:*** As mentioned, all the authors solely contributed to the research. The detailed contributions are listed below. We have added this list of details and roles of the authors in this study on the title page of the manuscript.

- *Added information: title page, Author contributions*

Hong-min Ahn: Data curation, formal analysis, investigation, validation, writing–original draft, and writing–editing.

Duck-Woo Kim: Conceptualization, investigation, validation, methodology, resources, project administration, writing–review, and editing.

Hyeon Jeong Oh: Investigation, resources, and methodology.

Hyung Kyung Kim: Investigation, resources, and methodology.

Hye Seung Lee: Investigation and resources.

Tae Gyun Lee: Data curation and validation.

Hye-Rim Shin: Data curation, validation.

In Jun Yang: Data curation, validation.

Jeehye Lee: Methodology, validation.

Jung Wook Suh: Methodology, validation.

Heung-Kwon Oh: Investigation, validation, methodology, resources, writing, review, and editing.

Sung-Bum Kang: Investigation, validation, methodology, resources, writing, review, and editing.

3) (In Abstract) Please write a brief about the tests used for statistical analysis

- ***Response:*** The statistical analysis that was used was added in the METHODS section of the abstract. We used the Cox proportion regression model for the multivariable analysis to identify the recurrence-related factors.

- *<Correction> Amended paragraph: Abstract – METHODS, page 3*

  This retrospective, single-center, observational cohort study included patients who underwent surgery for stage I–III CRC between January 2009 and December 2019. Patients with *KRAS* mutation status confirmed by molecular pathology reports were included. The relationships between clinicopathological characteristics and individual codon-specific *KRAS* mutations were analyzed. Survival data were analyzed to identify codon-specific *KRAS* mutations as recurrence-related factors using the Cox proportional hazards regression model.

4) (In Abstract) Conclusion may be more comprehensive and represents the all findings of the study

- ***Response:*** We thank you for the insightful recommendation. We have realized that conclusion should be more comprehensive and represents the findings of the study. We also have realized a possibility of confusion on the conclusion in abstract since the "AIM" in abstract was written too broadly. The aim of this study was to evaluate the clinicopathological characteristics and prognostic value of *KRAS* codon 13 mutations. Therefore, our study's main result is that comparing with codon 12 mutation, the *KRAS* codon 13 mutation is less likely to serve as a prognostic factor in colorectal cancer.

- *<Correction #1> Amended paragraph: Abstract – AIM*

  This study aimed to evaluate the clinicopathological characteristics and prognostic value of codon-specific *KRAS* mutations, especially in codon 13.

- *<Correction #2> Amended paragraph: Abstract – CONCLUSION*

  This study provides evidence that *KRAS* codon 13 mutation is less likely to serve as a prognostic biomarker than codon 12 mutation for CRC in a large-scale cohort.

5) The Key words should be chosen based on Mesh terms

- ***Response:*** Thank you for the constructive comment. We had chosen the key words from the MeSH terms; however, they were not from the "Main Heading (Descriptor) Terms". We have changed the key words with the heading terms from the MeSH tree website (https://meshb.nlm.nih.gov/search).

- *<Correction> Changed Key words*

  Key words: Genes, ras; Codon; Colonic neoplasms; Rectal neoplasms

6) (In Materials and Methods) You may reveal the routine procedures of the colorectal surgery department as you referred to this center to use their data.

(In Materials and Methods) Please bring a concise and precise details about "Adjuvant/Neoadjuvant therapy and follow-ups" based on the nature of Methods and Materials. It may be explored more comprehensively in Discussion section.

- ***Response:*** Thank you for your pertinent comments on the "Adjuvant/Neoadjuvant therapy and follow-ups" section of our manuscript. The intention of describing the worldwide clinical

guidelines was to demonstrate that our routine procedure of the colorectal surgery department is systemic and evidence-based. Understanding your comments, we realized the excessive description made it confusing. Therefore, we deleted the descriptions of other clinical guidelines and summarized the details about adjuvant/neoadjuvant therapy in our hospital's routine procedures in a concise manner.

- *<Correction> Amended paragraph, MATERIAL AND METHODS - Adjuvant/Neoadjuvant therapy and follow-ups*

All patients who underwent colorectal surgery for curative purposes were recommended adjuvant therapy according to the pathological stage of the cancer. Patients with pathological stage III and high-risk stage II colon cancer are recommended adjuvant chemotherapy. In rectal cancer, patients with pathological stages II and III are treated with adjuvant chemotherapy after surgery. However, in patients with clinical T4 or positive nodes without distant metastasis, preoperative chemoradiation therapy is recommended with long-course radiotherapy (dose of 5040 cGy of radiation over 5 weeks; 28 fractions) combined with chemotherapy with 5-fluorouracil/leucovorin or capecitabine.

7) (In Materials and Methods) Please indicate which tissue sample. Do you mean samples from cancerous tissues?

- ***Response:*** Yes, the tissue sample referred to the cancerous tissue from the surgical specimen. We have amended the sentence to clarify the meaning.

- *<Correction> Amended paragraph: MATERIALS AND METHODS – Data collection*

KRAS mutations were identified from formalin-fixed, paraffin-embedded cancerous tissue obtained from surgical specimens. After deoxyribonucleic acid (DNA) extraction from the tissue, the exons 2 and 3 of the *KRAS* gene were separately amplified by polymerase chain

reaction (PCR) using optimized PCR reagents and primers. Codon-specific *KRAS* mutations were identified by pyrosequencing (PyroMark Q24 Mdx, QIAGEN, Hilden, Germany). MSI status was also evaluated using formalin-fixed tissues during surgery. PCR with five markers (*BAT26, BAT25, D5S346, D17S250, D2S123*) followed by fragmentation assay (ABI-3130xl, Thermo Fisher Scientific, Massachusetts, USA) was performed to identify the MSI status.

8) (Table 6, in Disscussion) Not to be revealed in discussion section.

(Table 6) It is a novel application of the literature in original research. You may indicate the table in the text and discuss about that in Materials and Methods and discussion Sections.

Response: Thank you for your insightful comments on Table 6. At the beginning of this study, we reviewed previous studies on codon-specific *KRAS* mutation in colorectal cancer and realized that a large cohort study should be needed to understand the minor codon mutations, such as codon 13 or 61. Compared with the study from Japan in 2019, we have analyzed with 10 times larger cohort with long-term follow-ups. Compared with the study from China in 2019, twice the number of patients were analyzed. Most of these previous studies concluded similar results, such as the *KRAS* codon 12 mutation in colorectal cancer is correlated with poor oncologic outcomes. One study included codon 61 in their analysis; however, the number of patients with KRAS codon 61 mutation was only four, which was insufficient to analyze. We made these contents into a Table to show readers an overview of our study's large cohort.

Since the contents of Table 6 were already written by text and complemented in the Discussion section, we eventually deleted Table 6. However, according to the mixed comments about Table 6, we would like to ask if Table 6 seems inappropriate for our manuscript.

ᔓ *<Correction> Amended paragraph: DISCUSSION*

Among the 2,203 patients who underwent curative surgery for stage I–III CRC, the incidence of codon-specific *KRAS* abnormalities was, respectively, 27.7%, 9.1%, and 1.3% for patients with *KRAS* codon 12, 13, and 61 mutations. Only 9.3% (205/2,203) recurrences were observed during the 5-year follow-up period. To our knowledge, this study is based on the largest scaled cohort that has ever analyzed not only the oncological impact but also the clinicopathological characteristics of codon-specific *KRAS* mutations in patients with CRC. Most previous studies have reported similar results for *KRAS* codon 12 mutations, but not codon 13, in CRC as a poor oncological factor[3, 6, 9, 11, 17]. Despite the minimal oncological effects of minor *KRAS* mutations, such as in codon 61, the data obtained were sufficient to gain statistical power, supporting previous findings that *KRAS* codon 61 mutation is not associated with the clinicopathological features of CRC[18]. An earlier study in a Japanese cohort also identified *KRAS* codon 12, but not codon 13, as an independent risk factor for tumor recurrence in stage I–III CRC. While their results supported the utility of *KRAS* codon 12 mutation as a poor prognostic factor, the correlation between codon-specific *KRAS* mutations and clinicopathological characteristics could not be validated because of the small sample size [17]. In the present study, we analyzed the largest sample group of patients, which provided not only results complementing earlier studies on *KRAS* mutations in CRC, but also additional information on correlations with clinicopathological characteristics and prognostic factors for individual codon-specific *KRAS* mutations.

9) Please revise this part (In Conclusion).

ᔓ ***Response:*** Thank you for the comments. We have re-written the sentence to clarify the meaning that we had originally intended. The phrase “both of which affected the clinical characteristics of CRC patients” made a confusion on the conclusive sentence. Thus, we have deleted that phrase to make our conclusion clearer.

ଓ *<Correction> Altered paragraph: Conclusion*

Most of the *KRAS* mutations in our study involved *KRAS* codons 12 and 13. Notably, *KRAS* codon 12 mutation was significantly associated with pathological features closely related to cancer recurrence and had a poor prognostic impact in patients with MSS tumors, or those located in the colon but not in the rectum. Given its irrelevance to pathological features and recurrence, we propose that *KRAS* codon 13 mutation is less likely to serve as a prognostic factor for CRC.

10) The references should be revised and use up to dated references. Please replace old references with new studies. Of course, you will consider the status and impact of new references in the main text.

ଓ ***Response:*** Thank you for your thorough review. We agree that some of our cited references are old studies and have revised and added up-to-date references as follows.

ଓ *<Correction> Added references*

3 Wan XB, Wang AQ, Cao J, Dong ZC, Li N, Yang S, Sun MM, Li Z, Luo SX. Relationships among KRAS mutation status, expression of RAS pathway signaling molecules, and clinicopathological features and prognosis of patients with colorectal cancer. World J Gastroenterol 2019; 25(7): 808-823 [PMID: 30809081 PMCID: PMC6385012 DOI: 10.3748/wjg.v25.i7.808]

7 Fan JZ, Wang GF, Cheng XB, Dong ZH, Chen X, Deng YJ, Song X. Relationship between mismatch repair protein, RAS, BRAF, PIK3CA gene expression and clinicopathological characteristics in elderly colorectal cancer patients. World J Clin Cases 2021; 9(11): 2458-2468 [PMID: 33889611 PMCID: PMC8040173 DOI: 10.12998/wjcc.v9.i11.2458]

11 Asawa P, Bakalov V, Kancharla P, Abel S, Chahine Z, Monga DK, Kirichenko AV,

Wegner RE. The prognostic value of KRAS mutation in locally advanced rectal cancer. Int J Colorectal Dis 2022; 37(5): 1199-1207 [PMID: 35484252 DOI: 10.1007/s00384-022-04167-x]

39 Tonello M, Baratti D, Sammartino P, Di Giorgio A, Robella M, Sassaroli C, Framarini M, Valle M, Macri A, Graziosi L, Coccolini F, Lippolis PV, Gelmini R, Deraco M, Biacchi D, Santullo F, Vaira M, Di Lauro K, D'Acapito F, Carboni F, Giuffre G, Donini A, Fugazzola P, Faviana P, Sorrentino L, Scapinello A, Del Bianco P, Sommariva A. Microsatellite and RAS/RAF Mutational Status as Prognostic Factors in Colorectal Peritoneal Metastases Treated with Cytoreductive Surgery and Hyperthermic Intraperitoneal Chemotherapy (HIPEC). Ann Surg Oncol 2022; 29(6): 3405-3417 [PMID: 34783946 DOI: 10.1245/s10434-021-11045-3]

40 Formica V, Sera F, Cremolini C, Riondino S, Morelli C, Arkenau HT, Roselli M. KRAS and BRAF Mutations in Stage II and III Colon Cancer: A Systematic Review and Meta-Analysis. J Natl Cancer Inst 2022; 114(4): 517-527 [PMID: 34542636 PMCID: PMC9002292 DOI: 10.1093/jnci/djab190]

11) (Table 3) Please clarify why these data are missed.

ᘓ ***Response:*** Thank you for the comments on the blank in Table 3. Before the multivariable analysis was performed, we selected covariables from the univariable analysis. The covariance input criterion was $p < 0.1$ in the univariable analysis and the "rule of thumb" also was applied in selecting covariables for the multivariable analysis. Notably, only 12 variables matched the covariance input criterion $p < 0.1$, and we have added codon 13, and 61 as covariables even though they did not match the criterion. Therefore, only 14 covariables were used in multivariable analysis, which made blanks in the Table. We put a hyphen ("-") in the blank, and the annotation at the bottom of Table 3.

ᘓ <*Correction*> *Amended Table 3*

Table 3. Univariable and Cox regression analyses of *KRAS* mutations for determination of recurrence-related factors

	Recurrence			Multivariable Cox regression analysis**			
	Absent* (n=1,998)	Present* (n=205)	p-value	HR	95% C.I. Lower	95% C.I. Upper	p-value
Age (years)							
<65	933 (46.7)	93 (45.4)	0.716	-	-	-	-
≥65	1,065 (53.3)	112 (54.6)		-	-	-	
Sex							
Male	1,143 (57.2)	121 (59.0)	0.616	-	-	-	-
Female	855 (42.8)	84 (41.0)		-	-	-	
BMI							
<25 kg/m²	1,266 (63.4)	142 (69.3)	0.094	-	-	-	-
≥25 kg/m²	732 (36.6)	63 (30.7)		-	-	-	
ASA score							
1~2	1,802 (90.2)	185 (90.2)	0.980	-	-	-	-
3~4	196 (9.8)	20 (9.8)		-	-	-	
Cancer location (1)[a)]							
Right-sided	577 (28.9)	58 (28.3)	0.860	-	-	-	-
Left-sided	1,421 (71.1)	147 (71.7)		-	-	-	
Cancer location (2)[b)]							
Colon	1,376 (68.9)	127 (62.0)	**0.043**	1			
Rectum	622 (31.1)	78 (38.0)		1.053	0.718	1.545	0.791
Preoperative CEA							
<5.0 ng/ml	1,607 (80.4)	140 (68.3)	**<0.001**	1			
≥5.0 ng/ml	391 (19.6)	65 (31.7)		1.158	0.849	1.579	0.354
Diverting stoma							
No	1,568 (78.5)	138 (67.3)	**<0.001**	1			
Yes	430 (21.5)	67 (32.7)		1.874	1.260	2.787	**0.002**
T stage							
T0-2	659 (33.0)	17 (8.3)	**<0.001**	1			
T3-4	1,339 (67.0)	188 (91.7)		2.620	1.479	4.641	**0.001**
N stage							
N0	1,230 (61.6)	56 (27.3)	**<0.001**	1			
N1-2	768 (38.4)	149 (72.7)		2.001	1.399	2.861	**<0.001**
MSI status							
MSS	1,680 (84.1)	186 (90.7)	**0.037**	0.855	0.342	2.138	0.738
MSI-low	143 (7.2)	10 (4.9)		1.284	0.643	2.566	0.479
MSI-high	175 (8.8)	9 (4.4)		1			
Tumor size (cm)	4.3 ± 2.4	4.9 ± 2.1	**<0.001**	0.997	0.927	1.074	0.944
Lymphatic invasion							
No	1,493 (74.7)	113 (55.1)	**<0.001**	1			
Yes	505 (25.3)	92 (44.9)		1.324	0.977	1.793	0.070
Vascular invasion							
No	1,615 (80.8)	119 (58.0)	**<0.001**	1			
Yes	383 (19.2)	86 (42.0)		1.578	1.164	2.139	**0.003**
Perineural invasion							
No	1,211 (60.6)	58 (28.3)	**<0.001**	1			
Yes	787 (39.4)	147 (71.7)		1.684	1.194	2.376	**0.003**
Harvested LN	45.3 ± 21.2	44.9 ± 21.4	0.705	-	-	-	-
Metastatic LN	1.2 ± 2.6	3.3 ± 4.5	**<0.001**	1.028	0.995	1.061	0.095
***KRAS* Codon 12**							
Wild-type	1,459 (73.0)	133 (64.9)	**0.013**	1			
Mutation	539 (27.0)	72 (35.1)		1.399	1.034	1.894	**0.030**

***KRAS* Codon 13**							
Wild-type	1,809 (90.5)	193 (94.1)	0.088	1			
Mutation	189 (9.5)	12 (5.9)		0.637	0.350	1.160	0.140
***KRAS* Codon 61**							
Wild-type	1,975 (98.8)	200 (97.6)	0.176	1			
Mutation	23 (1.2)	5 (2.4)		1.950	0.790	4.812	0.147

HR, Hazard ratio; C.I., Confidence interval; BMI, Body mass index; ASA, American Society of Anesthesiologists; CEA, Carcinoembryonic antigen; MSI, Microsatellite instability; MSS, Microsatellite stable; LN, Lymph node

*Results are reported as mean ± standard deviation or as number (percent).

**No values indicated variables do not match the covariance input criterion ($p < 0.1$ in univariable analysis)

a) Right-sided: from the cecum to distal 2/3 transverse colon; Left-sided: from the splenic flexure to rectum

b) Rectum: below the pelvic inlet (an imaginary line drawn from the sacral promontory to the pubic symphysis)