Reviewer1：

Please explain the meaning of the association between patient's age and HER2. What is the scientific background for the association?

The authors need to consider tumor sidedness but not tumor site as a prognostic marker. It has been reported that there is significant difference in HER2 positivity between right colon vs. rectum [Oncotarget. 2017 Sep 21;8(49):86356-86368]. Please discuss the previous report regarding tumor biological difference according to tumor site [Clin Cancer Res. 2018 Mar 1;24(5):1062-1072].

1. Please explain the meaning of the association between patient's age and HER2. What is the scientific background for the association?

Response:

In this study, it has been proved that positive HER expression as measured by IHC confers a worse prognosis in those patients 65 years old or younger with tubular adenocarcinomas. While there was no correlation between HER2 status and age in several previous studies of gastric cancer (Gan To Kagaku Ryoho. 2015 Oct;42(10):1289-91). That may be due to different types of cancer and the result also needs further confirmation.

2. The authors need to consider tumor sidedness but not tumor site as a prognostic marker. It has been reported that there is significant difference in HER2 positivity between right colon vs. rectum [Oncotarget. 2017 Sep 21;8(49):86356-86368]. Please discuss the

previous report regarding tumor biological difference according to tumor site [Clin Cancer Res. 2018 Mar 1;24(5):1062-1072].

Response:

Thankful for your suggestion, we will supplement the results, instead of the site, about differences between right/left-sided tumors. And we will also discuss the finding.

Reviewer2

Introduction Please mention that HER2 is a predictive factor for treatment with trastuzumab in both breast and gastric cancer. 2. Immunohistochemical analysis You state: ”HER2 IHC scores of 2+ and 3+ were considered as being “HER2 positive”, while IHC scores of 0 and 1+ were considered as being “HER2 negative”. Please state that this is different compared to the scoring criteria for gastric cancer. Please see World J Gastroenterol 2014; 20(16): 4526-4535. Results Were there any differences observed between the colon cancer and the rectal cancer populations. Please describe. Discussion a. You state: ”No tumors with HER2 IHC scores of 1+ showed evidence of HER2 gene amplification by FISH”. Based on the analyses of only 10 samples with IHC1+ it is difficult to state this. For the TOGA trial (Lancet 2010; 376: 687-697) in gastric cancer a relatively large proportion of patients with IHC0 and IHC1+ score were FISH+. Please discuss this in relation to your study and moderate your statement of no evidence of HER2 amplification in the

IHC1+ group. b. You state: “Our study also indicates that there is a high concordance between HER2 IHC 3+ staining and HER2 gene amplification in colorectal adenocarcinomas.” For the IHC3+ population an agreement with FISH+ is expected to be in the range of 90-100%. Please explain that you only achieved an agreement of 83%. c. In relation to the discussion on the prognostic nature of HER2 positivity in CRC, please also discuss this in relation the information available for gastric cancer. Please see Int J Cancer 2012; 130: 2845-2856 and J Cancer 2012; 3: 137-144. d. You state that you found a high agreement between IHC and FISH, so please explain why you mainly only found a prognostic association for HER2 amplification and not for HER2 expression. e. Please discuss the differences observed between the colon cancer and the rectal cancer populations if any.

Answers to reviewer2

1. Introduction Please mention that HER2 is a predictive factor for treatment with trastuzumab in both breast and gastric cancer.

Response:

We are very thankful for your efforts to improve our research and accept the suggestions wholeheartedly, mentioning “HER2 overexpression/amplification is linked to trastuzumab response in breast/gastric cancers” in our manuscript.

2.

A: Immunohistochemical analysis You state: ” HER2 IHC scores of 2+ and 3+ were considered as being “HER2 positive”, while IHC scores of 0 and 1+ were considered as being “HER2 negative”. Please state that this is different compared to the scoring criteria for gastric cancer. Please see World J Gastroenterol 2014; 20(16): 4526-4535.

Response:

In gastric cancer, HER2 IHC scores of 3+ were considered as being “HER2 positive” and IHC scores of 0 and 1+ were considered as being “HER2 negative”. In addition, HER2 IHC scores of 2+ and HER2 gene amplification detected by FISH were considered as being “HER2 positive”. In our study, we considered HER2 IHC scores of 2+ and 3+ as being “HER2 positive”, while IHC scores of 0 and 1+ were considered as being “HER2 negative”.

B: Results Were there any differences observed between the colon cancer and the rectal cancer populations. Please describe.

Response:

As to clinicopathological features, we didn’t find any observably dif ferences between right/left-sided tumors in this study (p=0.514).

3. Discussion

A: You state: ”No tumors with HER2 IHC scores of 1+ showed evidence of HER2 gene amplification by FISH”. Based on the analyses of

only 10 samples with IHC1+ it is difficult to state this. For the TOGA trial (Lancet 2010; 376: 687-697) in gastric cancer a relatively large proportion of patients with IHC0 and IHC1+ score were FISH+. Please discuss this in relation to your study and moderate your statement of no evidence of HER2 amplification in the IHC1+ group.

Response:

Our study state:" No tumors with HER2 IHC scores of 1+ showed evidence of HER2 gene amplification by FISH". that is differently from the HER2 gene status of IHC 0 and IHC 1+ in gastric cancer for TOGA trail, maybe it is because of different tissues with different biological characteristics.

B: You state: "Our study also indicates that there is a high concordance between HER2 IHC 3+ staining and HER2 gene amplification in colorectal adenocarcinomas." For the IHC3+ population an agreement with FISH+ is expected to be in the range of 90-100%. Please explain that you only achieved an agreement of 83%.

Response:

In our study, the percentage of HER2 gene amplification in HER2 IHC 3+ staining patients is a little lower than the previous studies, but it may be improved for further study by increasing patient numbers.

C/D: In relation to the discussion on the prognostic nature of HER2 positivity in CRC, please also discuss this in relation the information

available for gastric cancer. Please see Int J Cancer 2012; 130: 2845-2856 and J Cancer 2012; 3: 137-144. You state that you found a high agreement between IHC and FISH, so please explain why you mainly only found a prognostic association for HER2 amplification and not for HER2 expression.

Response:

Sorry about our research, we didn't observe the significantly prognostic difference in all the colorectal carcinoma patients neither for HER2 amplification or for HER2 expression.

E: Please discuss the differences observed between the colon cancer and the rectal cancer populations if any.

Response:

It has been proved that the HER2 gene amplification was significantly different between right colon and rectum carcinoma. Unfortunately, we didn't find similar results, it perhaps related to different groups, HER2 testing methods, and tumor biological characteristics.