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Response to reviewer

COMMENTS TO AUTHORS

Zhang et al retrospectively reviewed a series of 125 patients with rectal cancer and demonstrated that omeprazole users had better prognosis in term of response and recurrence rates and disease-free survival. 1 This as a retrospective study, therefore the results may have been biased by several factors, including selection bias and sample heterogeneity. Sample size calculation has not been performed. Indeed, 125 subjects may be too few, and therefore the p values are very close to 0.05, thus limiting the strength of the study conclusions. 2 Page 4: "Abnormal pH gradients of tumor microenvironment is involved in tumorigenesis, tumor progression and drug resistance" this sentence is questionable, since PPI alter stomach pH, while colonic pH may be influenced by several other factors. 3 Continuous variables should be expressed as mean ± standard deviations (not error). 4 Patients received omeprazole at different doses and routes of administration. A subanalysis in terms of DFS and survival is necessary. 5. TNM was a stronger predictor of DFS (p=0.006) than EOG (p=0.044), therefore it is possible that, in comparison to tumor staging, use of omeprazole could be marginally significant in terms of prognosis. 6.Moreover it is strange that adjuvant CHT was not a significant predictor, therefore a discussion is needed. 7.PPIs are well known risk factors for infections in immunosuppressed patients (see Lambert AA et al, Plos One 2015). Authors should discriminate among deaths for cancer "per se" or for concurrent infections and correlate this finding to PPI use.

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Response to reviewer

Thank you for your kind review and we are really grateful to your comments that "omeprazole users exhibited better prognosis in term of response and recurrence rates and disease-free survival." we really appreciate your effort.

1. This as a retrospective study, therefore the results may have been biased by several factors, including selection bias and sample heterogeneity. Sample size calculation has not been performed. Indeed, 125 subjects may be too few, and therefore the p values are very close to 0.05, thus limiting the strength of the study conclusions.

Our study is a retrospective study, we recognized the shortcomings of the present study and discussed in the DISCUSSION part in the fourth paragraph as "Our study has its shortcomings. Although consecutive patients were included, it was a retrospective study. Besides, sample of the study was the relatively small."

For decreasing selection bias and sample heterogeneity, we enrolled consecutive patients and excluded patients lacking detailed medical record. The relative small sample of the study is result of strict including criterion for homogeneity because we excluded the patients radiation plan as conventional radiotherapy and Intensity modulated radiation therapy (IMRT), another reason for small sample is this is a single cancer center study. Really, the strength of the study conclusions is limited, we realized this shortcoming and explained in the DISCUSSION part in the fourth paragraph as "However, the effects of OME on CRT efficacy, tumor recurrence and patients' survival were the first investigated in present study, which would be helpful for randomized and controlled trials in future."

2."Abnormal pH gradients of tumor microenvironment is involved in tumorigenesis, tumor progression and drug resistance" this sentence is questionable, since PPI alter stomach pH, while colonic pH may be influenced by several other factors.

Indeed, colonic pH may be influenced by several other factors such as cell energy metabolism and hypoxia inducible factor. In present study,as introduced in **INTRODUCTION** part: omeprazole (OME) was validated that could inhibit the activity of Vacuolar type H+-ATPases (V-ATPases) [1-4](reference 17-20), which is a proton pump expressed on the membrane of endolysosomal organelles and on plasma membrane [5] (reference 5 in manuscript), and is reported that modulate tumor acidic microenvironment[6, 7] (reference 12,13 in manuscript). In *in vitro* studies, OME could re-sensitize drug-resistant cancer colon adenocarcinomas cell lines to cytotoxic drugs[8](reference numbered 26 in manuscript)。These studies results suggest OME may affect colorectal cancer cell behaviour via V-ATPases, however, this hypothesis need validate by further molecular biology studies. In present study, we clinically find that OME is associated with rectal treatment efficacy, which support our hypothesis.

3. Continuous variables should be expressed as mean ± standard deviations (not error).

According to reviewer's suggestion, we revised the expression of data as mean ± standard deviations(SD).

4. Patients received omeprazole at different doses and routes of administration. A subanalysis in terms of DFS and survival is necessary.

Patients received omeprazole at different doses and routes of administration. Indeed, Management data of omeprazolew in different doses is a key point to our study. We have tried to process subanalysis that to find effect of different OME administration mode, however, as could be found in table 1 as following, when divided patients group according to administered routes, samples of subgroups are very small (among 63 OME users, 7 patients only received OME orally, 47 patients only received OME intravenously, and 9 patients accepted OME both orally and intravenously), which would result in distinct sample heterogeneity. When we reviewed the related literature, we found oral administration could be transferred equally to intravenous

administration according to bioavailability. The related reference described detailed transfer methods are cited in the **Dosage of Omeprazole** part as "The reduction in gastric peak acid secretion after continuous oral administration of 20 mg OME once daily for six days was comparable with the effect of a single intravenous dose of 40 mg OME[9] (reference numbered 29 in manuscript). Thus, patients who received 20 mg OME orally at least once a day for six days and/or intravenous infusion of 40 mg OME daily were recognized as eligible OME users (EOU); otherwise, the patients were regarded as non-eligible OME users (non-EOU). Among the 125 patients, 63 patients met the criteria as EOU. Moreover, the bioavailability of oral enteric-coated omeprazole granules is initially low (approximately 35–40%); however, these levels increased to approximately 65% on repeated dosing [10-13] (reference numbered 30-33 in manuscript). Therefore, the oral dose of EOU was multiplied by 65% to convert to a dose comparable with the intravenous dose for the intention of equal drug bioavailability."

Table 1, Mean dose and duration of OME administered orally and intravenously

OME	Cases	administered dose (mg)				number of days OME was administered			
		mean±SD	95%CI	max	min	mean±SD	95%CI	max	min
Oral[a]	7	260.0±143.2	127.6-392.4	546	182	11.0±8.0	3.6-18.3	28	7
IV[b]	47	217.2±184.8	162.8-271.3	940	40	3.8±3.0	2.9-4.6	16	1
IV+Oral[c]	9	406.2±184.9	264.1-548.4	756	151	13.7±7.0	8.2-19.1	28	7

a: oral OME multiplied by 65%; b: OME received intravenously; c: oral OME multiplied by 65% plus OME received intravenously.

5. TNM was a stronger predictor of DFS (p=0.006) than EOG (p=0.044), therefore it is possible that, in comparison to tumor staging, use of omeprazole could be marginally significant in terms of prognosis.

In fact, we are very agreed with this opinion. Use of OME at routine dose aim at relieving the common side effects of the chemotherapy that could achieve a trend of anti-cancer effect is

presentable, because even the benefit of adjuvant chemotherapy is relative limited. In a pooled-analysis, fluorouracil (FU)-based chemotherapy was shown to increase the 5-year OS from 64% to 71% in a mixed group of node-negative (American Joint Committee on Cancer stage II) and node-positive (American Joint Committee on Cancer stage III) patients[14]. The benefit of treatment in patients with stage II disease has been less clear, and current American Society of Clinical Oncology guidelines do not recommend the routine use of adjuvant chemotherapy for all patients with stage II disease[15].

6. Moreover it is strange that adjuvant CHT was not a significant predictor, therefore a discussion is needed.

We accepted suggestion and revised our discussion in first paragraph of **DISCUSSION** as "Neoadjuvant CRT could greatly improve the anus save rate and decrease local recurrence rate in advanced rectal cancer management[16-19] (reference numbered 2-4,37 in manuscript). However, results addressing whether neoadjuvant CRT could improve survival are inconsistent[16, 19] (reference numbered 2,37 in manuscript). The results of the present study showed that CRT efficacy is a significant clinicopathological factor associated with DFS (p=0.031) and exhibits a favorable trend with OS (p=0.144), indicating that CRT could decrease recurrence and potentially benefit OS. The results of the present study suggest that CRT efficacy is a significant clinicopathological factor associated with DFS, and this result is consistent with previous studies [16-18] (reference numbered 2-4 in manuscript). The present study results suggest that CRT has a potential benefit in OS but is not a significant predictor. These results were consistent with the study by R Sauer et al[19] (reference numbered 37 in manuscript) but not the study of Calogero Cammà et al[16] (reference numbered 2)."

7. PPIs are well known risk factors for infections in immunosuppressed patients (see Lambert AA et al, Plos One 2015). Authors should discriminate among deaths for cancer "per se" or for concurrent infections and correlate this finding to PPI use.

We reviewed our database and revised the first paragraph of the **Survival between non-EOG and EOG** as "At the end of the study, 96 (76.8%) patients were still alive. The patients who did not survive all died of tumor-related death, and no patients died from PPI-related severe infection[20] (reference numbered 36 in manuscript)during the CRT treatment period."

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*

Sincerely yours,

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