Re: Submission of original article

Thank you very much for offering your expert review on our article titled “Impact of CAPOX or FOLFOX4 on Spleen Size, Platelet Count and Liver Function when Partnered Cetuximab as First-line Treatment for KRAS Wild-type Metastatic Colorectal Cancer”.

I am now providing my point-to-point response to the comments of the reviewers for your further expert review.

Reviewer 1:

Splenomegaly and thrombocytopenia are commonly attributed to vascular occlusion due to oxaliplatin. The cumulative dose of oxaliplatin in CAPOX w/o cetuximab was higher than that in FOLFOX w/o cetuximab. The dose intensity of oxaliplatin in CAPOX/FOLFOX was also higher than that in CAPOX/FOLFOX with cetuximab. It was difficult to accept this conclusion because there were significant biases.

Response: Thanks very much for your valuable comments. We admitted that the cumulative dose of oxaliplatin was higher in CAPOX without cetuximab as compared to FOLFOX without cetuximab. However the dose intensity of oxaliplatin between CAPOX w/o cetuximab and FOLFOX without cetuximab did not differ as shown in Table 2. In fact, we have also shown that cumulative dose of oxaliplatin did not correlate with splenomegaly and thrombocytopenia in both univariable and multivariable analysis which had already taken into account the difference of dose intensity and cumulative dose between these four groups of patients. Dose intensity and cumulative dose of oxaliplatin were added in both univariable and multivariable analyses which was described already in the Section “Statistical Analysis” from line 176 to line 184. Most importantly, multivariable analysis is the most statistically reliable and acceptable tool for factors which correlated with splenomegaly and
thrombocytopenia. Therefore we think there were no significant biases to present this finding since the most acceptable statistical method has been used. In fact we were confident to present this finding as univariable and multivariable analyses were employed.

**Reviewer 2: Minor revision**

The paper from Victor HF Lee and colleagues is aimed to evaluate the impact of chemotherapy (Capox or Folfox) with or without cetuximab as first line treatment for KRas wild type metastatic colorectal cancer on spleen size, platelet count and liver function.

It is known that oxaliplatin can cause hepatic sinusoidal obstruction and some papers have correlated this injury with increase morbidity and mortality after surgery for hepatic colorectal metastasis in patient treated with preoperative oxaliplatin based chemotherapy (Nakano et all, Ann Surg 2008; Vauthey JN et al, J Clin Oncol 2006).

As stated from the authors in the introduction, the increase in spleen size was recently proven to be a biomarker for such hepatic adverse event after oxaliplatin administration (Overman MJ et al, J Clin Oncol 2010). On the other hand, there are only limited data on the effect of Capox on the spleen size and its related complication as well as the protective effect of adding cetuximab to chemotherapy.

Therefore, the topic of this study could be of interest for Cancer Research Frontiers’ readers. Certainly the authors know that the main limitation of this study is the small number of subjects. However, the overall manuscript is well written, the introduction provides a good, generalized background of the topic.

The manuscript can be suitable for publication in Cancer Research Frontiers after the following minor criticisms are addressed:

**Minor Issues:**

- Material and Methods_Patients and Study Design section: could you specify that an informed consent from patients has been obtained?

**Response:** Thanks very much for your comments. The reviewer’s suggestion has been added in the revised manuscript on line 112.
• Material and Methods_Volumetric Evaluation of Spleen Size section: the statement in fifth line is not clear. Could You explain better what you mean?

Response: Thanks very much for your suggestion. Rephrasing of the description of volumetric evaluation of spleen size was made from line 155 to line 159, which should appear clearer to the reviewer.

• Results_Univariable and Multivariable analysis section: Could you create a table for the results of univariate analysis described in the text?

Response: Thanks very much for your suggestion. The results of univariable analysis was depicted in a new table (Table 3) and the original results of multivariable analysis was moved to Table 4.

• Results_Capox plus Cetuximab vs. Folfox4 plus Cetuximab section: What you describe as showed in Figure 2 does not correspond to the figure. Add appropriate figure or change the explanation of the figure in the text.

Response: Thanks really so much for pointing out this minor mistake. The text in the manuscript was modified as highlighted on line 230 and 231.

• Figure 1 a, b, c: add p value in the figure.

Response: p-values were added in Figure 1 already. Thanks very much for your advice.

• No mention of the NRas status is provided in the text.

Response: Thanks again for your valuable suggestion. The study was initiated in January 2010 and at that time NRAS was not proven a predictive biomarker for response to cetuximab. Even up till now, NRAS seems more a predictive biomarker for panitumumab rather than cetuximab. We therefore think that NRAS status should not alter our results to any extent.

Reviewer 3: Major revision
The effect of the association of cetuximab with traditional chemotherapy in the treatment of colorectal cancer is known, as the authors admit. What they emphasize is the impact of CAPOX/FOLFOX with or without cetuximab on on spleen size, platelet count and liver function in patients with KRAS wild-type metastatic colorectal cancer. The work is well done. However, because the use of cetuximab with the chemotherapy regimen does not avoid the onset of adverse effects related to the chemotherapy itself and, in addition, cetuximab is a self-financed drug and not all the patients can afford to buy it, in my opinion the final aim of the manuscript is quite weak.

The following issues need to be tackled:

- the authors should indicate: whether the patients have undergone a biopsy in order to stage the colon cancer; whether the patients have undergone any kind of surgery to resect the tumoral mass or part of it, or if all of them received therapy without surgery.

**Responses:** Thank you very much for your valuable comments. All patients had biopsy-proven colo-rectal cancer. It is not a routine clinical practice to perform biopsy of all metastatic nodules if they are confirmed radiologically on CT scan. So we do not think the metastatic nodules need histological confirmation if they were confirmed by our radiologists on imaging. An additional sentence was added on line 118 to 122 to confirm metastatic nodules by radiologists on CT scan.

- the text needs correction, also related to the English writing. I recommend a deep revision, concentrating on proofreading and editing.

**Response:** Thanks very much for your valuable comments. The revised manuscript has been edited and proofread by a native English speaker.

**Reviewer 4: Major revision**

Overall: This is an interesting report regarding the effect of treatment with CAPOX/FOLFOX +/- Cetuximab on spleen size as front line therapy for metastatic CRC. The results are interesting showing some association between use of capecitabine and higher rates of spleen enlargement, and with the use of cetuximab and lower rates of spleen enlargement. The main
limitation of the study lies in the low numbers of patients. Furthermore, there are manuscripts raising questions regarding the clinical significance of this finding. Additional comments are listed below for each section of the manuscript.

1. Title: The title does not represent the actual data within this paper. The study did not assess the impact of these treatments on platelet count or liver function. The main outcome was evaluation of spleen size and it’s effect on the other measures.

Response: Thanks very much for your precious comments. We have stated in the abstract and the main manuscript that splenic enlargement and splenomegaly correlated with platelet count and grade $\geq 1$ impaired liver function. Also dose intensity of capecitabine and cumulative dose of capecitabine correlated with grade $\geq 1$ impaired liver function as shown in the main text and Table 4. Overall we thought that the title is still appropriate to the contents of the manuscript.

2. Abstract:
   a. Major: The conclusions regarding cetuximab offering protection from splenic enlargement seems too strong for the data presented in the study.

Response: From our study, we really found that in both univariable and multivariable analysis that cetuximab correlated with less splenic enlargement and splenomegaly. In fact, the articles on the protective effect of bevacizumab (references 22 and 23 in our manuscript) had their study designs very similar to ours and they all concluded that bevacizumab had a protective effect from excessive increase in spleen. We believe that our conclusion is appropriate and neutral.

b. Minor: In the conclusions – the first sentence does not make sense: “CAPOX was associated with more splenic and …”

Response: Thanks very much for your valuable suggestion. This has been further modified on line 63 to line 65.

3. Introduction:
   a. Major:
- The introduction seems too long with some redundant sections. This includes the section describing the COIN study which could be significantly shortened.

**Response:** The revised manuscript was abridged according to the reviewer’s suggestion. Thank you very much for your comments.

- Would consider adding some data from the literature regarding the effect of splenic enlargement and why this would be a clinically relevant outcome to look at.

**Response:** Thank you very much for your valuable comments. We have already reported the complication of splenic enlargement by citing the article published by Overman et al (reference 20) from line 264 to line 271 of our revised manuscript. Also it is a well-known fact that splenomegaly can lead to thrombocytopenia.

4. Materials and Methods:
   a. Major:

   - This section again appears too long, and could be shortened (for example, there is no need to describe how KRAS mutation analysis was performed; this has no importance for this study).

   **Response:** Thanks for your comments. It has been abridged as shown on line 115 to 118.

   - The authors describe a “baseline liver impairment score” it is not clear how this was determined, is there any validation of this marker in the literature?

   **Response:** This comes from an article published by Twelves et al (reference 36 in the original manuscript and it has been now moved to reference 26 in the revised manuscript). For better clarity, the article is also cited on line 131 in the revised manuscript.

   - Thrombocytopenia was defined as PLT<150K. It is not clear why the NCI-CTCAE grading method was not used. The CTCAE grade 1 definition for low PLT is <=75K. It is not clear what is the clinical significance of a thrombocytopenia of <=150K.

   **Response:** Thank you very much for your comments. Thrombocytopenia was defined as <=150K, in accord with the previous paper published by Overman et al (reference 20 in our
manuscript) so as to allow favorable and consistent comparison. 150K is also lower limit of normal range in normal adults. So we think the definition of thrombocytopenia is appropriate. Reference 20 has been quoted after the sentence of definition of thrombocytopenia on line 142 of our manuscript.

- It appears that the change in the size of the spleen was evaluated using serial CTs. However the number of scans varied among patients. There is no timeline associated with the change in spleen size. It would be important to evaluate the change in all patients in a specific time point (like the first disease valuation scan). It is not clear from the text that that was done, and appears that the report is of overall change in spleen size among patients receiving various lengths of therapy.

Response: Thank you very much for the comments. Perhaps it is better if we modified the timing of CT scan as 9 to 10 weeks after the baseline then thereafter, as revised on line 155 to line 159 of the revised manuscript.

b.Minor:

- Are there any other studies that have used this method for evaluating the volume of the spleen. Would be helpful to add references to support the use of this method.

Response: Actually this is the standard and well-accepted method for radiation oncologists/clinical oncologists for measuring volume of any organ of interest, not necessarily the spleen. We have added one of our previous publications (reference 27 in the revised manuscript) on using this method for measuring the liver.

5. Results:

a. Major:

- Overall the section is somewhat confusing, would recommend re-organization of this section to discuss: patient’s characteristics and differences seen between the groups; the data regarding spleen enlargement; CAPOX vs FOLFOX; with/without Cetuximab; the effect of spleen enlargement on PLT and liver dysfunction; and uni/multivariate analysis. It seems that
statements regarding these various factors are scattered randomly throughout the text with repetitions of various facts.

Response: Thank you very much for the comments. The Results Section has been rephrased in the revised manuscript and should be more comprehensible. Distribution of splenic enlargement, splenomegaly, thrombocytopenia and impaired liver function was clearly depicted in Table 2.

- Only 43 patients continued treatment beyond CT time point 1 – this means about 60% of the patients progressed after 12 weeks of FOLFOX/CAPOX in the front line mCRC setting – this number seems very low. It is clear that there were other factors that contributed to this drop, these should be discussed.

Response: Thank you very much for your valuable comments. As stated in the manuscript, 60% of patients stopped treatment after CT time point 1 because of disease progression and intolerable toxicities. For better clarity, the proportion of patients with disease progression and intolerable toxicity mainly due to profound immunosuppression and oxaliplatin-related peripheral neuropathy were highlighted in the revised manuscript on line 196 to 201.

- In the uni/multivariate analysis – would separate the analysis of factors contributing to spleen enlargement to one paragraph and then factors contributing to PLT count/liver dysfunction to a separate paragraph.

Response: This has been further modified shown from line 245 to line 254 in the revised manuscript according to the reviewer’s suggestion. Thank for the comments.

b. Minor:

- Would add the actual numbers of each group of patients in the text.

Response: This has been further paragraphed already according to the reviewer’s suggestion. Thank you for your nice suggestion.

- The sentence: “Median increase in spleen size… after treatment…” Not clear what does after treatment means… see previous comment regarding the timing of spleen size evaluation.
Responses: As stated in response to your comments in 4a, the timing of scanning has been further specified as scanning at baseline and then every 9-10 weeks afterwards to make it more consistent. So the median increase in spleen size after treatment was revised as “Median increase in spleen size at CT Time Point 1” in the revised manuscript on line 203 to line 206. Thanks for pointing out the limitation of this sentence.

6. Discussion:

a. Major:

- The discussion is lacking discussion of major limitations of this study such as: sample size – which limits any definitive conclusions; the study design and the multiple factors that were out of the control of the investigators that affected treatment decisions; the issue of timing of spleen size evaluation;

Response: Thank you very much for the valuable comments. The limitations of the study were further elaborated on line 312 to 318.

- The authors discuss a difference noted between the groups with regards to oxaliplatin dose. This is an important point to discuss, however, the data shows that the group that got cetuximab had a lower dose intensity of oxaliplatin compared to their counterparts. Is it possible that this could explain the lower rate of splenic enlargement rather than the use of cetuximab?

Response: As mentioned in the text, dose of oxaliplatin was uneven among the groups as our study is not a randomized-controlled trial. However dose intensity and cumulative dose of oxaliplatin were incorporated into both univariable and multivariable analysis and they were not significant in both analyses. This valid point was also raised by reviewer 1 and it was addressed as well. Thank you very much for raising this valid point.

- The discussion regarding the difference in number of liver metastases is confusing without any clear conclusions made. Given the very low numbers in each category it is unlikely that this factor will have a major influence over the results, and it would be impossible to stratify based on that.
Response: Thank you so much for your comments. Similar to the above explanation, though there was uneven distribution of the number and volume of liver metastases, univariable and multivariable analyses revealed that they were not factors correlating with any of the splenic, hepatic complications and platelet count as stated in the statistical analysis that all these factors were analyzed in univariable and multivariable analyses. This has been elaborated on line 312 to 318 accordingly.

b. Minor:
- The sentence “Therefore, it was reasonable to speculate that capecitabine was the culprit for these splenic, platelet and hepatic sequelae”. Appears too strong, given the low numbers of patients, and the discrepancies noted between the groups. Furthermore, in the multivariate analysis capecitabine dose did not appear to affect PLT count as noted in table 3. The effect of capecitabine on liver function is known.

Response: Thank you very much for your suggestion. The sentence has been modified as “Therefore it was reasonable to speculate that capecitabine predisposed to these splenic and hepatic sequelae and subsequent thrombocytopenia secondary to splenic enlargement.” to make it less strong.

7.Tables/Figures:

a. Table 1:
- This table includes many factors that are not discussed in the text such as: median ccl; <=50ml/min; alcohol consumption, smoking, etc.), consider trimming down the table and making it less detailed.

Response: Thank you very much for your advice. We have trimmed down the table and remove some parameters e.g. baseline creatinine clearance <50ml/min and re-classify smoking and drinking status into fewer subgroups as attached.

- Not clear the difference between: liver only, liver and others, non-liver, and liver metastases.

Response: We have modified as attached in the revised Table 4. Thanks for the comments.
The section of number of liver metastases can be condensed to fewer categories.

**Response:** This has been further modified to only 0 liver metastasis, 1-5 metastases and >5 metastases in revised Table 4. Thanks very much for your comments.

- In the first part of the table add more text next to <50ml/min (GFR?)

**Response:** This has been added using Cockroft and Gault formula and revised in Table 4.

b. Table 2: the line “increase in net spleen size” - not clear, is that number of pt. that had a net increase in spleen size?

**Response:** This has been revised indicating number of patients with increase in net spleen size. Thanks for the comment.

The revised manuscript is now attached for your reference. Please kindly offer your expert review of my article.

I am looking forward cordially to your favorable reply soon.

Yours sincerely,

Victor HF Lee
Corresponding Author
Clinical Assistant Professor
Department of Clinical Oncology
Li Ka Shing Faculty of Medicine
The University of Hong Kong