Author's response to reviews

Title: Circulating lymphocyte is an important determinant of the effectiveness of preoperative radiotherapy in advanced rectal cancer

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Author's response to reviews: see over
Reply to reviewers comments

Reviewer 1,

Major Compulsory Revision

The paper presents and interesting idea that lymphocytes levels could be a predictor of response to RT in rectal cancer patient. This may be utilized in future as a non invasive technique to analyze response to therapy. However, there are a number of other factors that play role in the changes in lymphocyte levels and have not been taken into consideration by the authors. Lymphocytes are very prone to alterations even on minor stress to the human body. Factors such as chronic stress (here disease burden), metal deficiency, excessive sunlight exposure and most importantly age dependent variations. Further, as the timing of lymphocyte isolation varied in each patient (as noted by the authors) therefore, a conclusive analysis from this study is bit over-optimistic and extrapolatory in nature.

1. The authors should provide greater in-depth discussion on various factors affecting lymphocyte population.

   Basically, I agree to your suggestion that lymphocyte number is largely affected by various factors including age, nutrition and stress caused by treatment as well as disease itself. In particular, % lymphocyte was greatly reduced by radiation as shown in this text. However, in another way, this indicates that the % lymphocytes well reflect the total condition of the host to fight with cancer, which may be the reason why this value can be a good marker for tumor response to RT.

   As your suggestion, the lymphocyte number is largely affected by the timing of blood sampling during and after RT, and thus we can not draw the discrete conclusion. However, the % lymphocyte of pre-RT stage was not seriously affected by the date of blood sampling as shown in below figure and thus the can be a good prognostic marker. This was additionally described in Discussion.
Figure: Pre-RT % lymphocyte was not significantly different by the timing of blood isolation. (X axis in below figure means the days between blood sampling and the start of RT, P=0.22)

2. The authors must provide the platelet count, C-reactive protein and fibrinogen level in serum data in the manuscript to make in more comprehensive study. According to your suggestion, we add the data of CRP and Fibrinogen in Table 2 and Results.

Other minor Essential Revisions:
1. Numerous grammatical mistakes and spelling errors in abstract, results and discussion dampen the enthusiasm of the reader
1. Table 2 captions have errors.
   Those parts were all revised with the English check by a native.
Reviewer 2
Critiques:
1) The manuscript is marred with several typographical and grammatical errors.
   e.g.
   a. Page 2 line 4..adding should be changed to addition of
   b. Page 6 line 14….conversely spelled wrong.
   c. Page 8 line 1…as is unnecessary
      Those parts were all corrected.

2) Ratio and % counts are incorrectly used interchangeably adding to the confusion.
   % or percentage of lymphocytes was all corrected to “ratio”, and absolute number of lymphocytes was unified to “counts”.

3) Figures 2 and 3 do not add any value to the manuscript and can be removed.
4) The text linked to figs 2,3 do not indicate if any formal statistics was applied to pre and during treatment blood count values or are just purely descriptive.
   According to your suggestion Figure 2 was deleted. However, our data of sample size of more than 1000 can tell, at least, the tendency of lymphocyte reduction during RT which was not clearly described yet. Therefore, we would express the Figure 3 as new Figure 2, just as our finding.

5) True pathological and clinical CR were taken together as complete responders. The clinical CR represents 20% of all CR cases and they do not necessarily mean pathological completer response which might be a desirable surrogate since it positively correlates with overall survival.
   On this point, I really agree with your comments. Therefore, we showed the overall and disease free survival were significantly correlated with lymphocyte ration in Figure 1.

6) The conclusion in the abstract (page 2) and discussion (page 8 line 9)sections are far reaching and not consistent with data presented in the manuscript.
   The abstract and discussion were toned down.

7) Figure legend 1: Indicate it is pre-RT blood counts.
   This part was corrected as reviewers comment.
8) Table 1: size, CEA and circumferential extent should have greater than or lesser than signs before the numbers...it is unclear what is meant as it is stands. The numbers in table should be also be represented as % in parenthesis.

This was also corrected as reviewers comment.
The Ms by Kitayama et al., entitled "Circulating lymphocyte is an important determinant of the effectiveness of preoperative radiotherapy in advanced rectal cancer" describes clinical response to radiotherapy in locally advanced colorectal cancer based on evaluation of the relationships between circulating blood cell counts and RT effects to come to derive a prognostic value along with clinic pathological features. Their findings reveal the ratio of lymphocytes in WBC taken before RT was significantly greater in 15 CR cases as compared with those in non-CR cases. Patients with high lymphocyte percentages (25.7%) showed better outcome than the counterparts. Conversely, the ratio of neutrophiles was reduced in CR cases. The lymphocyte ratio showed an independent association with CR with multivariate analysis, and tended to be maintained at relatively high levels in CR cases. On basis of their findings they concluded that in RC patients, peripheral blood lymphocytes have a significant impact on the CR rate in response to RT implying the possibility that lymphocyte-mediated immune reactions are necessary for achieving complete eradication of tumor cells and that enhancement of immune function during RT may improve the effectiveness of RT for advanced RC.

As a reviewer, I recommend acceptance of this Ms subject to some minor corrections as indicated below. Under Introduction the authors should correct EGFR as Epidermal Growth Factor Receptor and not as Endothelial Growth Factor Receptor. Likewise, VEGF should also be corrected omitting the word 'cell'.

Thank you for your favorable comment. The parts were all corrected.