**Author’s response to reviews**

**Title:** Correlation of Aurora-A expression with the effect of chemoradiation therapy on esophageal squamous cell carcinoma

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**Author’s response to reviews:** see over
Thank you for your helpful comments. We have modified our manuscript in line with reviewer’s suggestions.

**Reviewer: Donald L. van der Peet**

(Minor Essential Revisions)

1. The study period is very long and the numbers of included patients low. Also indications for whether or not to operate are subjected to biases in this series. This might have an effect upon the results reported.

   **(Answer)** In this period, most of patients were treated with preoperative chemotherapy and we adopted CRT to relatively advanced patients after informed consent. Moreover we did not adopt CRT to the patients with synchronous or metachronous cancer in other organs. Therefore the patient’s number was limited. However, during this period, the patients were treated by same surgical team under same treatment strategies. Above explanation was added into Methods, Study groups and patient characteristics section.

2. Without legends Figures 1 and 2 are hard to interpret.

   **(Answer)** Figures are changed to the high power view photos.

3. How can the lack of long-term effect of Aurora A expression in survival be explained?

   **(Answer)** In the ESCC patients with treated with CRT, lack of Aurora-A expression might be a negative prognostic factor because of poor tumor shrinkage. However when patients have relapse disease in their follow up periods, it might be better prognostic factor. Therefore there was no significant differences on 5year-survival rates between patients with Aurora-A (+) and Aurora-A (-) tumors. Above description was added into discussion section.

4. There are many references to research done by this group. I would find it more compelling if work by other groups are cited as well.

   **(Answer)** We have reviewed the molecules known to correlate with response and/or prognosis to CRT in ESCC patients in reference 5, which cited many other works. We added some factors in background section.
Reviewer: Julie Izzo

(Major compulsory Revisions)
1. Delete "predictive value" from title and the manuscript all together: the data presented do not demonstrate prediction, but simple correlation.

(Answer) We deleted “predictive value” and changed them to other words and sentences. Firstly, the title was changed to “Correlation of Aurora-A expression with the effect of chemoradiation therapy on esophageal squamous cell carcinoma”, then Line 57 in Abstract, line 80 in Background, line 283-284 in Discussion, and line 291 in Conclusion was changed as shown.

2. Methods: please provide the details about the Aurora antibody from Cell Signaling used in the study, eg species and catalog number. Also provide evidence of validation for FFPE tissue. None of the Aurora A antibodies by Cell signaling has been validated for IHC, thus, considering the novel data provided by this manuscript, the authors need to demonstrate that the antibody is specific for the target protein.

(Answer) The Aurora antibody used in this study was Cell Signaling TECHNOLOGY # 3092 which is suitable for western blotting as well as immunocytochemistry. This antibody was also used for IHC of FFPE tissue in reference 10, Tong T, Zhong Y, Kong J, Dong L, Song Y, Fu M, Liu Z, Wang M, Guo L, Lu S, Wu M, Zhan Q: Overexpression of Aurora-A contributes to malignant development of human esophageal squamous cell carcinoma. Clin Cancer Res 2004, 10(21): 7304-7310. This information was added into method section and above paper was also referred.

3. In survival assessment: please perform multivariate analysis including variables such as stage, histopathology grade, aurora A staining (including both nuclear, and nuclear+cytoplasmic). The effect of Aurora A in survival (in particular in the context of data that are in contradiction with previous observations) could be "random", eg associated with proliferative nature of the tumor or other. A Cox regression analysis will help the reader to better understand the value of his marker (even if not statistically significant).

(Answer) On univariate regression analyses, pathological stage (pStage) and
histopathological grade significantly affected postoperative outcome (p=0.03 and 0.004, respectively), however nuclear and nuclear+cytoplasmic Aurora-A expression did not affect (p=0.3 and p=0.4. respectively). On multivariate analysis, pStage and histopathological grade were significant prognostic factors (p=0.008, hazard ratio=9.3 and 0.002, hazard ratio=5.6 respectively), however nuclear and nuclear+cytoplasmic Aurora-A expression were not significant prognostic factors (p=0.73, hazard ratio=1.3 and p=0.91, hazard ratio=1.2, respectively) (Table 4). Above paragraph was added into Result section and Table4 were also added.

4. Criteria for response in CRT only treated pts: the response assessment seems to focus on primary lesions. If the primary lesion showed response but novel lesions appeared, how was the pt classified? Please provide the number of pts that progressed on secondary lesions and evaluate the Aurora expression in those as well.

(Answer) Among 78 patients, 9 patients were judged as progressive disease. Among them 8 patients had enlargement of the tumor with 6 negative expression and 2 positive expression of Aurora-A, and 1 patient had novel distant lymph node metastasis whose Aurora-A expression was negative.

(Minor Essential Revisions)
5. How was the 10% cut-off established? Please describe in the manuscript?

(Answer) We have tested the significance value of correlation between CRT response and Aurora-A expression according to Aurora-A expression rates, such as 10%, 20%, 30%, 40%, and more than 50%, then best significance was obtained at 10%, which we set as cut-off. Above explanation was added to Methods, Immunohistochemical Examination section.
**Editorial requests**

1. Ethics - Please revise the Methods section of your manuscript to include the name of the ethics committee that approved your study. We recommend the following format? This study was approved by the ethics committee of [xxx] Hospital [or University]?  

   **(Answer)** We modified according to editor’s recommendation. “This study was approved by the ethics committee of Kagoshima University” was added to the beginning of Methods section.

2. Contact information - Please include an email address for each author on the title page.  

   **(Answer)** We added an email address for each author on the title.