Author's response to reviews

Title: Identification of colorectal cancer patients with tumors carrying the TP53 mutation on the codon 72 proline allele that benefited most from 5-fluorouracil (5-FU) based postoperative chemotherapy.

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Author's response to reviews: see over
September 15, 2009
Dr. Melissa Norton
Editor-in-chief
BMC Cancer

Dear Dr. Norton:

We thank you and the reviewers for reviewing our manuscript. We have revised the manuscript in light of the reviewer’s comments. I hope all these corrections made are satisfactory, and the revised paper will be published in “BMC Cancer”.

Sincerely,
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Response to the reviewer’s comments:

To Dr. Antonio Russo:

Thank you very much for reviewing our manuscript. In light of your comments, we revised the manuscript.

- Comment: problems in English.
  Response: The manuscript has been revised thoroughly by a native PhD scientist belonging to an English proofreading service.

- Comment: redundancy of the manuscript.
  Response: We checked and removed redundant contents throughout the text with the English editor as described below. In order to avoid confusions especially between descriptions about the simple TP53 gene codon 72 polymorphism and those about the polymorphism on the mutated allele or those about simple mutations, we had to leave some irritatingly repetitive expressions in the text.

  (1) In the “Background” section:

  #1: To focus on CRC, we removed a description about HNSCC from the second paragraph.

  #2: In the last paragraph, we simplified but left a description about HNSCC, because that is a well-established clinical evidence to show the significance of the TP53 codon 72 polymorphism in combination with the mutation, and there is no such report on CRC.

  #3: The last sentence of the last paragraph was deleted as indicated.

  (2) In the “Methods” section, we moved descriptions about detailed PCR conditions to the “Supplemental Information for Methods”, which was newly prepared for this revision.

  (3) In the “Results” section:

  #1: The last sentence of the part [TP53 codon 72 polymorphic status of the tumors] was removed.

  #2: From the part [TP53 mutations of the tumors], detailed descriptions such as mutation types or database information are moved to the figure legend for the Supplemental Figure 1.

  #3: To avoid anticipating descriptions for discussion and to make it readable, we reorganized and shortened the parts [TP53 codon 72 status with or without mutations and patient’s outcome] and [Significance of the codon 72 status of the mutated TP53...
on the patients' outcome after postoperative chemotherapy].

(4) In the “Discussion” section:

#1: We removed repetitive descriptions about the sample population.

#2: To focus on CRC, we removed a description about HNSCC from the beginning of the third paragraph.

- Comment: about limited size of the Japanese sample in the TP53 CRC International Collaborative study.
  
  Response: We mentioned about this point in the first paragraph, lines 17-19 of the “Discussion” part.

- Comment: about HNSCC report which is describing chemo-radiotherapy but not chemotherapy.
  
  Response: We emphasized the difference in the second paragraph, lines 9-10 of the “Discussion” part.

- Comment: about description for chemotherapy.
  
  Response: We substituted reagents with regimens.

- Comment: about conclusions, which could be erased.
  
  Response: To emphasize that our conclusions are under a limited condition, the word “found” was replaced by “raised a possibility” in the line 5 of the “Conclusions” part. The same correction was made in the “Abstract”. We also removed the last sentence of the last paragraph of the “Discussion”, and added a sentence “We need to collect more CRC cases to form definite conclusions” to the end of the “Conclusions” part.
Response to the reviewer’s comments:

To Dr. JT Plukker:

Thank you very much for reviewing our manuscript. In light of your comments, we revised the manuscript.

- **Comment:** about a report from Westra et al. in JCO 2006; 23:5635.
  
  **Response:** Westra et al. reported the importance of the *TP53* mutation status in predicting outcome of Stage III colon cancer patients with 5-FU based chemotherapy and described that the wild-type *TP53*-tumors gain survival benefit from adjuvant chemotherapy. We referred the report in “Discussion” section, line 11-12. In the present study, we focused especially on the codon 72 polymorphic status of the mutated allele of *TP53* in relation to postoperative chemotherapy.

- **Comment:** about the heterogeneous patient group, the poorly defined clinical information, different chemotherapy regimens, and taking no account of nodal involvement as well as L/N ratio.
  
  **Response:** The indication is quite right. First, we added clinical information as listed below.

  #1: The present study did not include hereditary tumors, and we added a word “sporadic” in line 4 of the page 5.

  #2: Tumor site information was provided by adding a column of “Site” in the Table 3.

  #3: Other clinical information including age and tumor size was also added in Tables, and described it in the “Results” part.

  #4: We added a column of “Lymph node metastasis” in the Table 3, and also added a description about the evaluation of lymph node metastasis as “The lymph node metastasis was significantly increased with the *TP53* mutation (p<0.05, chi-square test; Table 3)”, lines 27-29 of the “Results” part.

  #5: Description about the chemotherapy reagents were substituted with regimens, and rearranged like “The 5-FU based regimens included tegafur and uracil (UFT) following 5-FU: leucovorin (LV)/5-FU (RPMI regimen), 28 cases; LV/5-FU/CPT-11 (FOLFIRI/IFL), 11 cases; LV/UFT-based regimens, 55 cases” in the ”Method”, lines 12-14.

  #6: Each sample size of patients who received postoperative chemotherapy becomes too small to evaluate the survival when we sub-classified them according to the tumor
location, the nodal involvement status, and details in the chemotherapy regimen. We would like to carry over this important point to our future study. In stead, we described this limitation of our work in several parts of the text, such as “The cancer type, chemotherapeutic reagents and a combined radiotherapy can be listed as different points in the HNSCC clinical investigation.” in lines 11-12 of the page 11, “This result is not conclusive because we did not have a sufficient number of patients to compare in each Dukes’ stage, especially in stage C with or without postoperative chemotherapy.” in lines 14-16 of the page 11, ” Our results raised a possibility that Dukes’ stage C CRC patients with tumors carrying TP53 mutation, especially the P72 allele, benefited most from 5-FU based postoperative chemotherapy, but we need to collect more CRC cases to form definite conclusions.” in lines 8-11 of the page 12.

- Comment: The small number of Dukes’ C tumors to drawn general conclusion.
  Response: The indication is quite proper, and we described this limitation of our work in several parts of the text listed above, especially in the “Conclusion” section, describing as “Our results raised a possibility that Dukes’ stage C CRC patients with tumors carrying TP53 mutation, especially the P72 allele, benefited most from 5-FU based postoperative chemotherapy, but we need to collect more CRC cases to form definite conclusions”.
- Comment: What were the indications not to treat all patients?
  Response: Although we recommended the adjuvant chemotherapy to all Dukes’ C patients, 26 patients did not agree the chemotherapy. These circumstances were described in the “Method” subheading of “Patient population and tissue specimens”.
- Comment: about association between the TP53 codon 72 and age of onset, MLH1/MSH2 mutation, and tumor size.
  Response: The age of onset and tumor size were not related to the codon 72 polymorphism. We added columns of “Age” of tumor onset and “Size” of tumors into the Table 2 with statistic evaluation. Correlation between MLH1/MSH2 mutation and the codon 72 polymorphism of the mutated TP53 allele is very interesting. We would like to grapple with this interest in our next study.