Author's response to reviews

Title: Promoter methylation and large intragenic rearrangements of DPYD are not predictive of severe toxicity to 5-fluourouracil-based chemotherapy in gastrointestinal cancer

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Author's response to reviews: see over
Editor-in-chief
Melissa Norton, MD

Porto, July 06, 2010

Dear Dr. Norton

Please consider the revised version of our manuscript now entitled, “Promoter methylation and large intragenic rearrangements of DYPD are not implicated in severe toxicity to 5-fluorouracil-based chemotherapy in gastrointestinal cancer patients,” for publication in BMC Cancer.

We thank you for the thoughtful comments of the reviewers which enabled us to significantly improve the manuscript.
We revised the manuscript according to their comments, as detailed below.

Reviewer #1:
Major compulsory revisions:
- The title was changed
- Details about the MLPA and QMSP methods were added in the Methods section
- We agree with the Reviewer that the immunohistochemical analysis is not critical for the aims of the study, nor does it provide additional relevant data. Thus, following the suggestion of Reviewer #2, we removed all immunohistochemical data and figures from the manuscript.
- We re-phrased the sentence, to incorporate the Reviewer’s point of view.
- We also re-phrased according to the Reviewer’s suggestions.

Minor essential revisions and discretionary revision: all were performed as indicated

Reviewer #2:
Major compulsory revisions:
1) A more detailed description of the mutational analysis was added;
Table 1 is now mentioned in the Material and Methods section.
Concerning the toxicity of patients harbouring different *DPYD* mutations, a sentence was added in the manuscript, mentioning the grade 4 toxicity presented by the patients.

2) A more detailed description of the QMSP analysis was added.
Reference [24] was replaced by [25].
Details regarding *DPYD* primers positions and the amplicon size were added.
Details about the PCR conditions and the serial dilutions for calibration were added to the manuscript; the legend of Figure 2 was modified to incorporate information concerning the dilutions. We also included in the figure the amplification curves of representative cases (all negative for methylation of *DPYD* promoter). Owing to the scarcity of DNA, it is not possible to perform bisulfite sequencing. However, we feel that this is not necessary because all cases tested negative and a recognized positive control (RKO cell line) amplified well, as expected. Thus, we do not believe that the assay is providing false negative results.

We did not incorporate the table providing methylation ratios because all tested cases were negative. However, we added to the text the value determined for RKO cell line methylation ratio (1075).

We re-phrased the sentence concerning the sensitivity and specificity of the QMSP assay to incorporate the Reviewer’s point of view.

3) Following the Reviewer’s suggestion, we removed all immunohistochemical data and figures from the manuscript.

Minor essential revisions: all were performed.

Editor’s comments
- The name of the institutional review board was added to the text.
- The abstract was structured according to BCM Cancer guidelines.
- The e-mail addresses of all authors was included.
- An Author’s contributions section was added.

We hope that the manuscript is now suitable for publication in *BMC Cancer*.
Thank you for reviewing our work.

Sincerely,

Carmen Jerónimo

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