Author's response to reviews

Title: Is there a role for the quantification of RRM1 and ERCC1 expression in Pancreatic Ductal Adenocarcinoma?

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Author's response to reviews: see over
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Dear Editor-in-chief

This is a cover letter for our manuscript titled as “Is There a Role for the Quantification of RRM1 and ERCC1 Expression in Pancreatic Ductal Adenocarcinoma?” The revised manuscript and the responses to the reviewers’ comments are enclosed for your review. All changes could be tracked using the “Track Changes” tool of the Microsoft Word® software and all the modifications are highlighted or in different font color. Also enclosed is a point-by-point statement on how we have addressed the reviewers’ comments and suggestions.

If you have any questions regarding the contents or the format of his manuscript, please do not hesitate to contact me. All the authors have no conflict of interest to disclose.

Yours sincerely,

Matias E Valsecchi, MD, MS
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Dear Reviewers and Editor,

Thank for your constructive suggestions. We have revised the manuscript as per your comments and we wish to thank the reviewers for their helpful comments. Please find below are our response to your comments.

Reviewer # 1:

1) – “The authors state that mRNA samples for ERCC1 and RRM1 were classified as “Low” or “High” expression according to cut off values pre-established by Response Genetics [...] This problem is clearly stated by the authors in the discussion (“ Other sources of variability could be related to the cut off values used for PCR detection ….”) while no solution was done to solve it.”

The cut-offs were established by Response Genetics using the same methodology that was applied to develop cut off values for NSCLC. The pancreatic ductal adenocarcinoma (PDA) cases that were tested to establish those cut off values were not included in the study and a clarifying sentence was added to the materials and methods section. We agree with the reviewer regarding the fact that changes in the cut off values may have impact in the final results; however we believe that, at this point, finding new values to validate those results are beyond the scope of our study.

2) – In the present manuscript the median survival of the entire cohort was 18 months which is less than the observation arm of Conko-001. This fact demonstrate the limitations of the present retrospective analysis and while cannot be resolved it should be mentioned in the text.

We agree with the reviewer and we have included a new paragraph in the discussion section acknowledging this fact. The paragraph reads: “However the median OS in our cohort (18 months) was slightly inferior to that previously reported in randomized clinical trials (i.e.: in Conko-001, 22 months) [26]; this could be attributed to the more heterogeneous population of our study in comparison to one that could be observed in prospective and controlled clinical trials.”

As stated in the new paragraph, we believe that part of the inferior survival observed in our population may be due to the patient heterogeneity (i.e.: not every patient completed chemotherapy as planned and we also included stage III and IV patients) which, as mentioned by the reviewer, is a consequence to the retrospective nature of this study.

Reviewer # 2:

1. a.) – How were the cutoff values determined for IHC for RRM1 and ERCC1? Did it follow any other trials? No trials are referenced.

The values used for the interpretation of IHC stains were previously published and the references are included in the manuscript. This is based on what was previously published for NSCLC. For RRM1 and the immunoreactive score please see Lee JJ et al [Lung Cancer 2010; 70 (2):205-10] manuscript reference # 23. For ERCC1 intensity see Olaussen et al [N Engl J Med 2006, 355(10):983-991] manuscript reference # 12.
1. b.) – The sample size is inadequate for DFS (64 patients) and DFS should be omitted from the report because OS is reliable in pancreatic cancer. It is also only 42 patients (cf. Table 4A) for OS using RTqPCR.

   We agree with the reviewer regarding the fact that OS is a more significant indicator in pancreatic cancer and we have stated that in the Discussion section. In fact, our conclusions are based mainly on the results obtained from OS. We also agree that 64 patients may cause our study to be underpowered to detect a real difference in DFS. However, since DFS was part of our original primary endpoint we prefer to include this data as it is, but acknowledging this limitation. We have added a sentence in the Discussion section referring to the DFS which reads: “As expected, this raises some concerns regarding selection bias in our cohort and conclusions regarding this matter should be analyzed cautiously and confirmed in future studies.”

1. c.) – Additionally it is problematic to have both, patients with and without chemo after resection as reflected in the HR of 13.09 after RTqPCR in table 4A and HR=3.04, i.e. the most significant parameter, by IHC score (Tbl 4B).

   We agree with this point. Actually clarification of this issue will improve the quality of our paper and we thank the reviewer for astutely pointing this out. We have included a table (new table # 5) showing the results of both, OS and DFS, but have only included the 82 patients who received the full course of chemotherapy. Unfortunately, for a myriad of reasons (toxicities, patient’s performance status, patient’s preferences, etc) 12 patients did not receive the full course of chemotherapy as originally planned. We have included this information in the results section. Importantly, the exclusion of these 12 patients did not produce any significant change in the overall estimates or our conclusions. The only value that showed a trend toward significance was RRM1 measured by qPCR (mentioned in the Discussion section) but this trended in the opposite direction to what is expected (low RRM1 expression should correspond with better OS). This may be attributed to the low number of patients tested as “high” (N = 7).

2) – “… The number of patients not receiving chemotherapy is not given in materials and methods or results. Only the discussion mentions that 87% of the patients had gemcitabine. However it is not clear how high this percentage was in the DFS calculations.”

   Please see previous answer (1.c) addressing the issue of patients not receiving chemotherapy.

“…The question of the significance of ERCC1 would have to be investigated in trials where Gem-platinum doublets have been utilised in pancreatic cancer (as suggested by the work of Kamikozuru et al. Int J Oncol 2008). Thus, ultimately this study only confirms the absence of prognostic and predictive significance of ERCC1 in patients treated with no or non-platinum chemotherapy found in NSCLC.”

   We agree with the reviewer and we have included a new sentence in the Discussion section acknowledging this point. The sentence reads: “Yet it seems to be clear that ERCC1 expression levels do not have any prognostic value in this patient cohort who
did not receive platinum based chemotherapy. Future investigations should explore the significance of ERCC1 in patients receiving platinum based regimens.”

3) – “… Pre-clinical work suggests that high RRM1 levels cause resistance to Gem, that Gem induces the expression of RRM1 and that siRRM1 knockdown sensitises cells to Gem. Overall the available clinical data in NSCLC are largely in favour of an association between RRM1 expression level and quality of response to gemcitabine. […] The multivariate PCR data in Table 4A (42 patients) from this manuscript point into the same direction whereas the IHC data obtained from more patients (n=91) do not. This finding needs to be critically interpreted as it is unexpected in the light of the NSCLC work and compared with the Akita paper.”

We agree with the reviewer in both of these points. There is indeed a vast amount of pre-clinical investigations in pancreatic cancer (mainly in cell lines) as well as pre-clinical and clinical evidence in NSCLC supporting a role of RRMM1 levels in predicting response to gemcitabine. However, at the time that our manuscript was first drafted, there were no clinical studies, with the exception of the one by Akita and colleagues, supporting the role of RRM1 in pancreatic cancer. Sometimes pre-clinical studies do not necessarily correlate with clinical outcomes. One notable difference between pancreatic and NSCLC in regards to gemcitabine sensitivity is that NSCLC seems to be intrinsically more sensitive to gemcitabine than PDA. This could, in part, explain why RRM1 levels may have a more predictive role in NSCLC. Future studies with different biomarkers or most likely a combination of biomarkers might enhance our understanding of this issue. Moreover, one of the papers mentioned by the reviewer (Kim R et al) also found no correlation between RRM1 mRNA levels and OS and DFS, in terms of prognosis, in patients with PDA. We have included these points in the revised Discussion section and we have also discussed the difference between this and the Akita’s paper results.

“… Similar to the lung data the data on pancreas also point to an impact of RRM1 protein levels/mRNA levels on gemcitabine response. The conclusion that RRM1 levels do not have a predictive or prognostic value in resected PDAC patients is difficult to justify in the light of the limitations of the presented work including treatment inhomogeneities (especially Gem vs no Gem) and the IHC cutoff value dilemma. The limitations of this study need to be discussed more critically in this report.”

We have explained these three points above: 1) – The potential differences between the lung and the pancreatic data (see previous response) 2) – The exclusion of those patients who did not receive chemotherapy (1.c) 3) – The issue of ICH cut off values (1.a). We have also tried to expand the Discussion section to be more comprehensive and underscore these limitations. Lastly, we have also cited in our revised version most of the papers referenced by the reviewer.

Thank you.