Reviewer's report

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

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**Reviewer:** Thomas Brunner

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This is a study on an important topic, the pharmacogenomics of gemcitabine in pancreatic cancer to help to better understand the reasons for resistance to the most important type of chemotherapy for this drug focusing on RRM1 and ERCC1. The conclusions drawn from the results should be better justified in the light of the current literature.

**Major Compulsory Revisions:**

1. There are several methodological weaknesses in this report:
   a. How were the cutoff values determined for IHC for RRM1 and ERCC1? Did it follow any other trials? No trials are referenced.
   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.
   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).

2. ERCC1 was shown in NSCLC to be prognostic and predictive when platinum based chemotherapy is employed but not when non-platinum or no Cx is used. Therefore, the negative result in this study is not surprising. 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. The high ERCC1 expression correlated with longer survival in the Akita study from the literature is counterintuitive and probably biased by small sample size. Additionally, only 40% of the patients had chemotherapy with gemcitabine. In this light the conclusion that ERCC1 has no prognostic value in pancreatic cancer is wrong: It should read that it is absent in patients not undergoing platinum-based 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 NSLC.
3. RRM1 is the more interesting molecular component in pancreatic cancer among the two which were studied because of its role in gemcitabine sensitivity. As ERCC1 it is also very interesting for the treatment of NSCL and findings in this disease need to be discussed in the light of the findings from pancreatic cancer in this report. 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.

Overview of PDAC and RRM1:
Kurata et al. Int J Cancer; 15 pancreatic cancer cell lines; RRM1 levels correlate with sensitivity to GEM
Kim R et al. Cancer 2011; 84 pts with surgery; qRT-PCR n.s.
Fujita H et al. Neoplasia 2010; 70 pts, 40 with Gem mRNA;Low RRM2 has longer DFS in the gem treated patients
Tanaka M et al. Cancer 2010; 149 pts c gem-based CRT SNPs: RRM1 A33G C-27A; RRM1 SNPs related with PFS
Mitsuno M et al. Int J Oncol 2010; PDAC cell lines, Tranilast reduces RRM1; Tranilast sensitisises to Gem
Ohhashi S et al. AnticRes08; PDAC lines, siRRM1; Sensitisises to Gem
Nakahira S IntJCa 07; MiaPaCa-2 siRRM1; Sensitisation of gem resistant cells to gem
Giovannetti CR2006 102 patients, Mixed treatment groups; Longer time to progression with low RRM1 (n.s. OS)

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.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:

I declare that I have no competing interests