Author's response to reviews

Title: ABT-737 increases treatment efficiency of paclitaxel against hepatoblastoma

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Author's response to reviews: see over
Dear Prof. J. Neuzil,

Thank you very much for the opportunity to revise our attached manuscript entitled „The BH3 mimetic ABT-737 increases treatment efficiency of paclitaxel against hepatoblastoma” for publication in *BMC Cancer*.

In the following we give a point-by-point response to the concerns of Reviewer#1:

**Major comments:**

1. “Overlap of data already presented in a previous report”
   
   Data of viability assays were presented for a better comparison of the activity of ABT-737 in combination with Paclitaxel on HB with those on fibroblasts. To estimate the impact of the treatment on HB cells an additional assay quantifying active Caspase 3 was performed. The data were presented in Fig 1C. Dose dependent activation of Caspase 3 was observed during treatment with paclitaxel. This was increased by ABT-737.

   The suggested clonogenicity assay was also performed, however, the plating efficiency of the cell lines was very poor (less than 0.7%). Although the combination treatment with paclitaxel and ABT-737 suppressed the colony formation entirely, we do not publish these data, due to the biases introduced by the low plating efficiency.

2. “Statistical significance of presented data”
   
   Comparison of the tumor volumes in the group of paclitaxel and Paclitaxel + ABT-737 using the student t test revealed a p=0.038. In the multiple analysis the significance (p< 0.05) was not reached, although the mean volumes were quite different. This may be a result of the variance of the starting tumor volume. Now the relative tumor growth was calculated as a proportion of the initial tumor volume. Multiple comparisons of the groups revealed a significant enhancement of paclitaxel treatment by ABT-737. The data are shown in Fig 2B.
3. a) “Role of Bcl-2 in HB”
In our department we already described the effect of Bcl-2 on treatment of HB. SiRNA experiments revealed the impact of Bcl-2 on CDDP, paclitaxel, and other drugs (Warmann SW, Pediatric Blood Cancer, 2008). Based on this work, we now describe an in vivo application of the already presented concept.

3. b) “Toxicity”
Due to the ethical approval the number of mice was limited and extended toxicological analysis could not be performed. Histological analysis of liver tissue revealed a changed morphology after combination treatment, which was not found in the other groups. Now, we describe and discuss these findings in the manuscript.

Minor comments:

4. “Single agent activity”
We agree with the concern that ABT-737 is poorly efficient in most solid tumours except SCLC as a single agent. We changed citations and explain the rationale for the use of ABT-737 in HB.

Discretionary revisions / recommendations for improvement:

5. “Control (enantiomer)”
Using enantiomers as controls was also our point. We used enantiomers only at the highest concentration in the combination with some concentrations of paclitaxel. There was no evidence of changes in cell viability compared to paclitaxel alone. The data were not presented, as only few points in the graphs were available. We mentioned this now in the manuscript.

6. Title
Thank you for the suggestion. The title was now changed.

Thank you very much again for your suggestions. According to those we have revised the manuscript and feel it has gained relevance now to be considered for publication in *BMC Cancer*.

Sincerely,

Sorin Armeanu-Ebinger