Author's response to reviews

Title: ENMD-1198, a novel tubulin-binding agent reduces HIF-1 and STAT3 activity in human hepatocellular carcinoma(HCC) cells, and inhibits growth and vascularization in vivo

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Version: 5 Date: 6 June 2008

Author's response to reviews: see over
Dear Editors of BMC Cancer,

we are submitting our re-revised manuscript entitled “ENMD-1198, a novel tubulin-binding agent reduces HIF-1α and STAT3 activity in human hepatocellular carcinoma (HCC) cells, and inhibits growth and vascularization in vivo”, to BMC Cancer to be considered for publication as a research article.

We have done all effort to further improve the previous revised version, as requested by Reviewer #1 (Dr. Park). However, we are not able to recover cells or to perform additional staining analyses in a timely adequate fashion. We therefore hope that both Reviewer #1 and the Editor can accept our explanation/modifications, also in light of the comment by Dr. N. Mabjeesh, who is an expert in the field of 2ME2/HIF-1, suggesting “acceptance” of this article. We humbly ask for an editorial decision in this matter.

We thank Dr Park, also an exceptional HIF-1 expert, for the comments and we did make additional changes and responded to his suggestions.

Responses to reviewer #1 are as follows:

1.) We agree that the magnification of CD31 staining looks different, we therefore adjusted the images which initially were all taken with same magnifications. We have no explanation for this formatting error.

2.) We did absolutely not ignore this reviewer’s comment on checking HIF-1α activity in tumors and thus provided additional analysis of HIF-1α protein by Western blotting of tumor tissues in our last revision (Figure 6c). Staining may sometimes be nuclear and/or cytoplasmatic which is different to interpret and, in addition, tumor sizes affect this staining (mentioned in the reference). In addition mouse tissue is very challenging to stain for HIF-1α. We hope that the reviewer does accept this approach of validating HIF-1α by Western blot.

3.) The figure shows analysis of tumor tissue protein by Western blotting for Hif-1α, which is the kind of bands that is typical for HIF-1 analysis in tissue samples recovered from the freezer, as the reviewer certainly knows from his own experience.
We apologize for this quality, however, we will not be able to get prettier results within the set deadline for revision. Nevertheless, we are convinced that the difference seen should be obvious to the reader.

4.) Western blotting of HepG2 was omitted as suggested by the last reviewing process and replaced by better and new analyses with loading for actin (was requested). However, due to time limitations we only had recovered one cell line, but overall we certainly have demonstrated these findings in 2 cell lines, as mentioned in the text.

5.) We somehow do not understand this comment. The graph does already represent normoxic = constitutive values! And indeed ENMD-1198 just marginally affected constitutive VEGF expression, a special finding that we addressed in the discussion section.

6.) We have added this information in the text of Results section.

All authors concur with the submission of this manuscript and we assure that the material submitted for publication has not been previously reported and is not under consideration for publication elsewhere.

We appreciate your time in handling and reviewing our manuscript, and anxiously look forward to receiving your final decision.

Kind regards,

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