Author’s response to reviews

Title: Antiviral Treatment Perspective against Borna disease virus 1 infection in Major Depression: A Double-Blind Placebo-Controlled Randomized Clinical Trial

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Author’s response to reviews:

PHAT-D-19-00096 Revision

Authors’ response to reviewers

Response to Reviewer 1:

Reviewer 1 judged our study positively as being well designed and novel and had no concerns at all. The authors would like to thank Reviewer 1 for her encouraging evaluation.

Response to Reviewer 2:

Reviewer 2 acknowledged the major clinical finding of our study, namely the antidepressant efficacy and safety of amantadine over placebo in BDV-1 infected MD or BD patients. Reviewer 2 acknowledged the predictive value of pre-treatment infection markers, like BDV-1 antibodies, for clinical improvement, as well as anti-BDV efficacy of amantadine in vitro.

However, the most challenging issue raised some concerns, namely whether amantadine exhibited its antidepressant effect through antiviral action against BDV-1. We are therefore grateful to Reviewer 2, whom we know as recognized expert of BDV research, for his very useful suggestions and comments to improve the manuscript.
Point-by-point consideration (in the order they have been mentioned):

Major points

1. Discuss antidepressant vs. antiviral effect of amantadine

We found that high virus antigen load (PAG) and high antibody titres which express high virus activity and strong host immune competence, respectively, could predict a high clinical benefit by amantadine. Reviewer 2 is right in saying that how the drug supported a favourable outcome remained elusive given the known antidepressant capacity of amantadine. In our opinion, however, the stronger decrease of virus variables in the amantadine group suggested at least a combined antiviral/antidepressant effect (see also point 5).

- Anti-manic effect of amantadine in BDV-1 infection

There is a further argument supporting an antiviral action. Amantadine had been shown to have significant anti-manic effects in BDV-1-infected bipolar I or bipolar II inpatients which were completely outside any reported pharmacological properties. In fact, the opposite effect, namely worsening of manic symptoms, would have been expected.

Revised manuscript, discussion: We inserted a paragraph (new lines 566-573) and a new reference [61], additional lines 594-596, and a further paragraph (new lines 600-610) with a further new reference [63].

2. Discuss other antivirals without antidepressant effect

We are grateful to reviewer 2 for his request to include known anti-BDV-1 antivirals without antidepressant effects, like ribavirin and favipiravir, in the discussion, because this will considerably improve the antiviral pharmacological aspects of the manuscript. We addressed his request in great detail.

Revised manuscript, discussion: We inserted a new paragraph (new lines 620-640 and 661-663) on the nucleoside analogues ribavirin and favipiravir, and three new references [70-72].
3. **BDV-1 in vitro studies, infection model**

We agree with reviewer 2 that in vitro efficacy studies should consider that BDV-1 establishes a persistent infection. In fact, we used OL cells persistently infected with either the human strain BDV Hu-H1 or the laboratory adapted animal strain V to study dose-dependent inhibition of replication (reduction of virus titres) over a 2-months period. To further clarify the experimental design, the corresponding paragraph in Methods has been thoroughly revised.

Revised manuscript, methods: We re-wrote the in vitro study paragraph (inhibition of replication) in Methods (new lines 248-258).

4. **Original lines 410-413, correlation of infection and clinical variables**

We thank reviewer 2 to request a more detailed description on the rather complex correlation within and between infection variables and clinical outcome. All data of the statistical analysis calculating Pearson’s correlation coefficient were provided in Table 6. We focused on the first treatment period of 7 weeks as the primary response. To achieve sufficient statistical power, amantadine and placebo groups were analysed together. In accord to the high clinical effect size of amantadine over placebo, correlations could be mainly assigned to the amantadine group. Nevertheless, correlation data on separated patient groups were also provided (Fig. 7 a-d), indicating a clearly more pronounced decrease of infection variables in the amantadine group related to decrease of depression (HAMD reduction), as compared to the placebo group. This clarification has been integrated in the revised manuscript.

Revised manuscript, results: We re-wrote the corresponding paragraph in Results (new lines 416-429; 437-440).

5. **Original lines 566-570. Discuss pre-treatment values vs. BDV-1 reduction and clinical outcome**

We acknowledge that reviewer 2 raised an important point which require amendments. We need to better explain why and how infection variables contribute differently to infection outcomes. We evaluated infection through three interdependent variables, CIC, PAG and AB, which reflect the dynamic of BDV-1 activity in blood plasma of patients. High virus protein levels (PAG) indicate a strongly active infection, as high antibodies titres indicate a strong immune competence of the patient. Circulating immune complexes (CIC) are a two-sided variable which is largely driven by antigen as well as antibody levels, both of which drop when CIC levels increase. Therefore, CIC displayed no correlation to short-term clinical improvement within 7 weeks, but did so on the long run.
Against this background, it is an important outcome of our study that high PAG and AB levels in depressed patients were predictive of a favourable clinical outcome by amantadine therapy, also in terms of clinical practice. The clearly more pronounced decrease of infection in the amantadine compared to the placebo group suggested at least a combined antidepressant/antiviral effect of the drug.

Moreover, the antiviral in vivo efficacy of amantadine had been previously demonstrated independently of clinical effects in remitted BDV-infected patients with affective disorders, applying a double-blind placebo-controlled design over 14 weeks. Activity-related variables (PAG and CIC) were significantly reduced (p=0.028 and p=0.003, respectively), as well as antibodies (AB; p=0.007). We inserted a new paragraph in the revised manuscript to further discuss this complex issue and added a new reference [63].

Revised manuscript, discussion: We inserted a new paragraphs and a new reference [63] in the Discussion (new lines 600-610), also mentioned in the context of point 1.

Minor points

6. Original line 70, DALYs, definition

DALYs are defined as disability-adjusted life years. The term had been explained in the list of abbreviations, but needed, of course, to be defined when first mentioned in the text. We inserted the definition in line 71 of the revised manuscript.

7. Original lines 249-251, Methods, time-point to add amantadine

We used persistently infected OL cells (see also point 3 of authors’ response). The cultures were kept at different doses of amantadine from day 0 to day 60. The split ratio during this treatment period was twice a week.

Revised manuscript, methods: We re-wrote and complemented the respective part in Methods (new lines 248-258); see also point 3.

8. Original line 251, Methods, virus titration

The virus titre at different time-points was determined through cell lysates. Virus infectious units were expressed as focus forming units (FFU/mL) and visualized by a focus immune assay.

Revised manuscript, methods: Please see new lines 252-257.
9. Original line 442, define “additive infection values”

We thank reviewer 2 for this question. The wording was really unclear. What has been meant could be better defined as cumulative infection scores which were built from the sum of CIC + PAG + AB test results. After 12 months post-therapy, dropped HAMD scores were significantly correlated with the drop of these cumulative infection scores.

Revised manuscript, results: We revised the manuscript accordingly (new lines 454-457).

10. Original lines 641-642, alleviate conclusion

We agree with the suggestion of reviewer 2 and toned down the statement made under point 6 of the conclusion. The extent to which the antiviral capacity of amantadine contributed to its antidepressant activity remained elusive, although a combined antiviral/antidepressant effect was suggestive from our data.

Revised manuscript, conclusion: We changed the wording accordingly (new lines 701-703).

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Authors’ remarks to the Editor

Dear Prof. Gü müstekin,

We like to thank both reviewers for their encouraging and useful comments. Reviewer 2 raised important points which needed further clarification.

In our response to reviewers, please find our detailed point-by-point explanations and comments. In the revised manuscript, all points are thoroughly addressed, indicating the new lines where text has been changed or new text inserted.

In accord to requests of reviewer 2, five additional references have been inserted as well.

We think the revised manuscript could have been significantly improved, especially through the helpful comments of reviewer 2.

Finally, we like to thank you very much for your assistance and hope to receive a favourable answer soon.
Yours sincerely,

Prof. Detlef E. Dietrich, corresponding author,

Dr. Liv Bode, co-corresponding author