Author's response to reviews

Title: Using high titer West Nile intravenous immunoglobulin from selected Israeli donors for treatment of West Nile virus infection

Authors:

David Ben-Nathan (davidbn@013.net.il)
Orly Gershoni-Yahalom (orlyge@bgu.ac.il)
Itzchak Samina (isami@moag.gov.il)
Yevgeny Khinich (vaccine@moag.gov.il)
Israel Nur (israel@omrix.co.il)
Orgad Laub (orgad@omrix.co.il)
Ahuva Gottreich (ahuva@sheba.health.gov.il)
Michael Simanov (vaccine@moag.gov.il)
Angel Porgador (angel@bgu.ac.il)
Bracha Rager-Zisman (rager@netvision.net.il)
Nadav Or (nadav@omrix.co.il)

Version: 4 Date: 8 December 2008

Author's response to reviews:

December 7 2008

Bio Med Central Editor
BMC Infectious Diseases
Re: MS 8846492292163447

Dear Editor,

We have carefully read the comments and suggestions raised by the reviewers and the editor of our manuscript (MS: 8846492292163447) entitled "High titer West Nile IVIG from selected donors for treatment of West Nile infection" The manuscript was thoroughly revised according to these comments. We thank the reviewers and the editor for their constructive comments and we hope that the revised manuscript will be accepted for publication in BMC Infectious diseases.

Following the editors recommendations,

1. We have changed the title of the manuscript to read: “Using high titer West Nile Intravenous immunoglobulin from selected Israeli donors for treatment of West Nile virus infection.”

2. We give the full name of IVIG where it first appears in the text (page 2 line 4).

3. We clarified “OMRIX” in the abstract to “OMRIX Biopharmaceuticals, Israel” (page 2, line 8).

4. A list of abbreviations was added (page 16).
5. The manuscript was copy-edited by a professional English language editor.

Response to the specific points raised by the reviewers (by the order they appear in the reviewer’s reports):

Reviewer #1: Thomas Kreil

1. Methods, page 5, line 8:

Both i.p. and subcutaneous routes were used for administration of WNV in mouse challenge models. Essentially our goal was to induce systemic infection in the model animal as reported, for example, in our previous publication (Ben Nathan 2003). The i.p route has been also used for WNV challenge by other groups, including Bai et al (2005), JID 191:1148; Bourne et al, (2007) JVI 81:9100; Beasley et al, (2002), Virology 296:17 and others.

A relevant reference has been added to the text (page 6, line 2).

2. Methods, page 6, line 5:

It was shown in the reference provided by the reviewer (Niedrig M et al, 2007) and in the present submission (Table 1) that there is clear qualitative agreement between the ELISA and cell neutralization assays. As shown in Table 1, the ratio of antibody activity when tested by the two methods was around 10 (11.4 by ELISA and 9.6 by PRNT50). One of the outcomes of our work is thus the possibility of utilizing ELISA results for selecting plasma units for the production of WNIG. A relevant clarification paragraph has also been added to the Discussion (page 13, line 15-17).

3. Methods, page 6, line 8:

A list of abbreviation was added (page 16).

4. Methods, page 7, line 22:

A relevant statement was added in the Methods (page 8, line 10-11). The disease, however, is probably dependent on additional parameters, such as infectious dose and immune status of the host, so we believe that direct comparison to humans is problematic and highly speculative. Therefore, we prefer not to discuss this in the manuscript.

5. Results, page 9, line 15 and Discussion, page 13, line 12:

We accept the reviewer’s view about the qualitative analysis. The relevant paragraphs were omitted from the Results and discussion (pages 9 and 13).

6. Results, pages 11 and 14; Resistance of adult mice to WNV.

The relative susceptibility of mice to WNV probably depends upon different parameters, such as WNV genotype, infectious dose, the animal’s genetic background and the route of infection. According to our experience in the mouse model, we found that adult Balb/C mice (7-8 weeks old) are more resistant to a low dose of WNV (5-10 PFU), as compared to animals at 4-5 weeks. In any case, this does not change the relevant evaluation, since the Discussion refers to
immunosuppressed animals treated with dexamethasone which became very sensitive to a low dose of infection.

The relevant paragraph in the Results was clarified (page 11, line 19-20). The relevant paragraph in the Discussion was also modified (page 14, line 11).

7. Discussion, page 12, line 20:
The relevant paragraph was re-edited and clarified (page 12, line 19-22).

8. Discussion, page 13, line 11:
The statement is related to the protection experiment performed in mice. The paragraph was clarified accordingly (page 13, lines 6-15).

9. Discussion, page 14, line 11:
The relevant paragraph was clarified (page 14, line 11-20).

10. Tables and Figures, Table 1:
Unfortunately, we were not able to test the IVIG-US lot by the PRNT50 assay. However, the ELISA results, with all its limitation (see also comment #2 above), clearly show that the level of specific antibodies to WNV in the US IVIG is very low and probably lower than in the regular IVIG-IL product. This is an expected result, as mentioned in the text, and we believe that the results in mice are in good agreement with this point. The major aim of this study was to compare the efficacies of IVIG-IL and WNIG. The inclusion of IVIG-US was mainly to introduce continuation and a common denominator to our previous study (Ben Nathan, 2003).

11. The legends to all tables have been extended to include more details.

12. The whole text was thoroughly inspected for spelling and grammar errors and edited according to the required format.

13. Methods, page 6, line 9:
All of the IVIGs used in the study (IVIG-IL, IVIG-US, and WNIG) were 5% IVIG produced by OMRIX, Israel. A clarifying comment has been added (page 6, line 6-15).

14. Results, page 9, line 22:
The results of viral titers in blood and brains of the animals were clarified (page 9, lines 18-23).

15. Results, page 10, line 19:
The relevant titers were included in the text (page 10, lines 18-20)

16. Result, page 11, line 11:
In all experiments, the animals were followed for at least 21 days. A clarification comment was added to the Methods section (page 8, lines 11 and 18)
17. Discussion, page 12, line 5:
A reference to other animal models has been added (page 12, line 12).

18. Discussion, page 12, line 18:
The results of other relevant publications are now included in the Discussion (page 12, lines 15-19).

19. Figure Legends:
Figure legends were clarified, as suggested.

Reviewer #2: Raymond Koski

1. The text was edited in light of comments #1, #2 and #3, as required. (page 7, line 8; page 3, line 24; page 11, line 10)

4. We agree that a negative control produced from negative/low anti WNV antibody titer plasma will serve well in this study. However, the protective effect of non-specific stimulation of the murine immune system by IVIG is very limited, if at all, as demonstrated by Planitzer (2007) and Ben Nathan (2003).

5. The specific efficacy of each IVIG batch (produced from US donors, as well as Israeli donors) is expected to be different, depending on the history of the specific donors (Planitzer, 2007). See also our response to the first Reviewer, comments #2 and #10, above.

6. We agree with the comment on our conclusion regarding the clinical potential of IVIG. The text in the Discussion has been modified to clarify this point (page 15, line 6).

Sincerely

Dr. David Ben Nathan
Corresponding author