Reviewer's report

**Title:** High titer West Nile IVIG from selected donors for treatment of West Nile infection

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**Reviewer:** Thomas Kreil

**Reviewer's report:**

“High titer West Nile IVIG from selected donors for treatment of West Nile virus infection”

In the study, WNV antibody levels of the Israeli donor population were screened by ELISA and blood donations with high arbitrary WNV antibody titers (>100 AU/ml) were pooled in order to produce an IVIG with high WNV antibody titer (WNIG).

In a small animal model, WNIG, IVIG derived from Israeli donors (IVIG-IL) and IVIG derived from US donors (IVIG-US) were shown to be protective against an otherwise lethal WNV challenge. Further, a protective effect of WNIG even during active encephalitis was shown.

The authors conclude that the use of WNIG for therapy and prophylactic applications might improve the current treatment options, a notion that clearly deserves further consideration. At the same time though, passages of the manuscript lack clarity and it also contain a few unsubstantiated conclusions. The following comments need to be addressed before the revised manuscript may be recommenable for publication:

**Major Compulsory Revisions**

**Section Methods**

**Page 5, Line 18:**

To substantiate the weight of their results, the authors need to justify their choice of an i.p. challenge route, instead of a subcutaneous WNV challenge which would mimic the natural infection of humans more closely.

**Page 6, Line 5:**

“This product was prepared during 2006 from Israeli plasma that was pre-screened by ELISA for specific anti WNV antibodies”

The authors refer to a correlation between the WNV ELISA and neutralization assays, based on only two corresponding data sets (table 1). Also, while the used WNIG lot was also screened by PRNT50, the results are not provided in the article.

Particularly as others have previously shown that for WNV ELISA does not
correlate with virus neutralization (Niedrig M. et al., 2007), a statistical analysis of the correlation needs to be provided.

Also, the authors do not discuss the well-known serological cross-reactivity within the Flaviviridae family nor provide any specifications for the ELISA assay. In general it is hard to see how the ELISA might substitute for a functional WNV assay (i.e. neutralization) for any clinical / commercial purposes.

Page 6, Line 8:
Introduce the abbreviation for IVIG from Israeli source (IVIG-IL)

Page 7, Line 22:
For the Immunoglobulin treatment of mice, different doses were used (0.01-8mg/mouse). When compared to the treatment recommendations for patients with primary immunodeficiencies (200-600mg/kg), this is a rather low dosage. Please discuss the relevance of the chosen experimental dosage as compared to the clinical situation in humans.

Section Results

Page 9, Line 15:
“…7-15 AU were sufficient to give 87-94% protection. However, in the case of IVIG-US, 7 AU gave 63% protection, possibly pointing to qualitative differences between the specific antibodies in US and Israeli donors.”

and Section Discussion Page 13, Lane 12:
“Our results suggest that there are qualitative difference between WNV specific antibodies from US and Israeli donors. We showed that 7-15 AU of antibodies from Israeli donors were more potent than 7 AU of antibodies originated in US donors.”

Both these statements are based on minimal ELISA data only, which are clearly insufficient to support a conclusion of such general nature. If the authors wish to substantiate their claim, then a direct comparison of the in vitro neutralization capacity and the in vivo protection of IVIG preparations needs to be performed, and then discussed based on reults.

Page 11, Line 15:
“Adult mice are relatively resistant to WNV…”

Mice have long been used as a WNV challenge model, and other investigators have shown that they are rather susceptible to WNV infection, with LD50s as low as 1pfu after peripheral injection (Beasley et al., 2002; Papin et al., 2005) and high mortality rates (> 90%).

The authors need to discuss their findings in light of these other results.

Section Discussion

General: The Discussion section is rather long, even somewhat confusing, and
discusses many things but not - the results generated in the presented study: a significant reduction in length as well as a focus on the results of the author’s own research is requested.

Page 12, Line 20:
Death is not a severe symptom of encephalitis (as stated on page 12, line 20). Severe encephalitis can lead to or result in death, pls clarify

Page 13, Line 11:
“…IVIG produced from Israeli donors and at least one log increase compared of IVIG produced from US donors”
The authors need to provide more precise information, i.e. the WNV neutralization titer of the specific material used, etc.

Page 14, Line 11:
“The natural resistance of adult mice to WNV infection…since the development of specific immunity is longer than the critical time…” The argument made here is not correct. Mice as laboratory mice have long been used as a model for WNV infection and a high mortality is observed in mice (> 90%).
The authors need to substantiate their statements versus earlier published findings by other groups.

Section Tables and Figures
Page 22, Table 1:
Anti-WNV antibodies for the IVIG-US (K30G370) lot was not tested using a functional assay, e.g. PRNT50. The information is crucial though for an appropriate assessment of the data provided in the manuscript.

General
The descriptions of tables are rather limited: more detailed information would clearly increase legibility, pls consider.

Minor Essential Revisions
General:
The authors could add line numbering, to support reviewing the suggested corrections.

Also, there are numerous spelling errors (e.g. page 3, line 3: intrevenous) and grammar mistakes throughout the text (e.g. page 5, line 2: ‘…at the age of 4-5 weeks…’; page 4, line 9-11: ‘…IVIG preparations……were protective…’; page 6, line 23: ‘…the plates were then blocked’, page 10, line 13: ‘The induction of de-novo…’; page 13, line 21: ‘…as many antibodies…’ etc.), which should be easy to correct using word processing.

Finally, use of different font sizes (Table 3 and Table 4) is confusing.
Section Methods
Page 6, Line 9:
Please provide a more detailed description for the IVIG-US Lot (Batch #K30G370) used, e.g. which IgG concentration was used?

Section Results
Page 9, Line 22:
“no virus was detected in blood of mice treated with WNIG”
The authors need to provide the data referred to.

Page 10, Line 19:
“The surviving animals developed high levels of anti WNV antibody titers…”
The authors need to provide the data referred to.

Page 11, Line 11:
The survival of mice was monitored for how many days?

Section Discussion
Page 12, Line 5:
“…protection and treatment of infections associated with WNV was studied using several animal models” The authors should briefly describe the other used animal models, and may want to include reference [19].

Page 12, Line 18:
“….it can be speculated that IVIG will have beneficial effect in infectious diseases such as WNV…”
Beyond the speculation the authors need to broaden their discussion, to include the results obtained by a number of recent publications that show the beneficial effect of IVIG in the treatment of WNV infections.

Figures
Place Figure legends before Figures
Instead of using the ‘Figure legend’ function in Excel to describe each sample it would be more easy to give details of the different samples in the Figure legend, including units for the IVIG and WNIG concentrations used.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

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

**Declaration of competing interests:**

Dr. Thomas R. Kreil is employed by Baxter BioScience, a company that has conducted research into the use of IVIG for the potential prevention and treatment of virus infections, including West Nile Virus.