Reviewer's report

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

**Version:** 3  **Date:** 9 October 2008

**Reviewer:** Raymond Koski

**Reviewer's report:**

This study reports an important, predictable advance in the development of a potential passive immunotherapeutic for treatment of WNV infections, for which no specific therapeutic is approved for human use.

The authors designed this study to demonstrate that an IVIG with a higher WNV ELISA titer is more effective in protecting mice from a WNV challenge. The methods are clearly described and appropriate, and produced significant data supporting the authors' hypothesis. In particular, the data in Table 2 and Figure 2 support the conclusion that IVIG from selected donors is more effective in protecting mice from an otherwise lethal dose of WNV.

**Minor Essential Revisions:**
1. In ELISA methods, change “PnPp substrate” to “pNPP substrate.”
2. The Background statement “Currently there is no effective therapy or vaccine for WNV infection” should be revised to “Currently there is no effective therapy or human vaccine for WNV infection.”
3. The Therapeutic Efficacy Results statement “After demonstrating a delayed therapeutic effect of treatment…” should be changed to “After demonstrating delayed mortality after treatment with...”

**Discretionary Revisions**
4. The experimental design would have been better with a negative control IVIG preparation, e.g., IVIG prepared from the 90% of the Israeli plasma units with <100 AU/ml. For example, injection of matched doses of negative control IVIG in the high dose experiment (Table 3) would have revealed the portion of the antiviral effect due to non-specific stimulation of murine immune system by injecting 2-8 mg of human immunoglobulin. A statistically significant difference between treatment with WNIG and the negative control IVIG would indicate that enrichment of WNV-specific human antibodies provided the therapeutic benefit.

5. IVIG-US had different WNV animal model protective results in cited publications. The PRNT titer for the IVIG-US used in this study should be determined and presented in Table 1. A positive PRNT result would support the authors’ conclusion that the 63% protection provided by 2 mg/mouse IVIG-US (Table 2) results from a low level of WNV-neutralizing antibodies. A negative
PRNT result would suggest that the 63% protection is due to a non-specific IVIG effect unrelated to WNV neutralization.

6. It should be clarified that i.p. administration of WNIG within a few hours of i.p administration of the WNV challenge (Table 2, Table 4, Figure 1) does not provide a model for treatment of active, disseminated WNV infection. The conclusion that WNIG offers “great potential for controlling active infection even in the CNS” is based on Table 3 and Figure 2 data, which lack negative control IVIG groups.

**Level of interest:** An article of importance in its field

**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:**

I am a co-inventor on patent applications relating to antibody therapy of flavivirus infections.