Author's response to reviews

Title: West Nile Virus Neuroinvasive Disease: Neurological Manifestations and Prospective Longitudinal Outcomes

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Author's response to reviews: see over
Dear Neil:

Thank you for the thoughtful and timely reviews of our manuscript. We have addressed each issue of the Reviews as outlined below point-by-point with our responses in italics, including notations of changes in the text as described:

Reviewer 1:

Major Points:

1. ….. In this kind of a prospective evaluation of clinical outcome, it is not possible to ignore the fact that some of the subjects did receive specific immunoglobulin as a means of therapy and some did not. One cannot help but wonder if there had been any differences in mortality rates, secondary infections, any side effects that can attributed to the hyperimmune globulins administered, the contents and dosage of the preparations, etc. Thus, it can be misleading to combine the clinical data from all cohorts, leaving out the therapy as a parameter. Moreover, it is explained in the manuscript (page 7) that "... the results related to the therapeutic interventions are in preparation", suggesting that some variations, either in outcome or the severity of the symptoms were observed. The authors need to provide at least some data and statistical comparisons among the groups or, even better, combine all data in a single manuscript. As recommended by the reviewers, efforts to publish the “negative” results of the therapeutic clinical trial are ongoing. In order to justify merging the 3 treatment cohorts for the neurological outcomes analyses presented in this manuscript, a brief summary of the results of the safety and efficacy analyses have been added (pgs.14-15 in text).

2. The laboratory diagnosis of WNV is not clear. Which assays were employed for the detection of IgM (capture or standard assays? which brand?). Is IgG investigated in all subjects? Have both serum and CSF were available for testing? Has intrathecal synthesis of specific antibodies been noted? Have positive results in serum samples been confirmed by virus neutralization? Which PCR assay was used for viral RNA detection? The definition of a confirmed case must also be provided. At the time that this protocol was written in 2002, detection of WNV IgM (preferably in CSF) was considered the diagnostic test of choice for acute WNV infection. No additional confirmatory laboratory testing (such as plaque reduction neutralization test, which was not widely available) was required by the protocol. All serologic studies were performed in the Clinical Virology Laboratory at the University of Alabama at Birmingham (UAB) using the ELISA methodology provided by Focus Technologies. Some subjects were allowed to enroll on the basis of positive IgM test performed at the study site, but confirmation of IgM positivity by the central UAB laboratory was required for the subject to remain enrolled. Among the subjects included in this analysis, 55/55 were WNV IgM-positive in serum and/or CSF; 53/55 were WNV-IgG positive in serum. A confirmed case was a subject who met the protocol inclusion/exclusion criteria and had a positive assay for WNV IgM from CSF and/or serum. WNV IgG assays were not performed on CSF. As
expected, WNV was detected in blood by PCR in only 3 of the 55 subjects; as the PCR data are not relevant to this discussion, mention of the PCR assay has been deleted. As all of the subjects in this study were enrolled in the US in the midst of a large WNV epidemic, infection with dengue or yellow fever (not endemic in the US) is an exceptionally unlikely cause of the observed neuroinvasive disease. Detailed travel histories were obtained from all subjects, but were not significant (data not shown). Infection with a different flavivirus causing serologic cross-reactivity with the WNV assay cannot be excluded with complete certainty (pgs. 7, 8, 9, 12-13 in text).

Minor Points:

- (Abstract line 4,12 and 14) some grammar and syntax errors were observed in the abstract. This has now been corrected as suggested (pgs. 3-4 in text).

- The conclusion section in the abstract does not provide clear statements as conclusions, but include comparison of initial and late neurologic deficits or findings. These should be moved to the results section and conclusions should be rewritten. This has now been corrected as suggested (pgs. 3-4 in text).

- (Page 14): "Neurologic outcome(s)" has been used as both a main and a subtitle, which is confusing. This has now been corrected (pg. 16 in text).

- (Table 3 ve 4): These tables include all capital letters, which is hard to read. The term "Polio" should be either explained as a footnote or be replaced with asymmetric limb paresis or weakness. This has now been corrected as suggested (pgs. 32-33 in text).

Reviewer 2:

1. Page 4 “Background”. The Authors should adjourn the epidemiology of WNV in the USA to 2012 (and possibly in a less defined way for 2013) since these data are currently available on the CDC web site. (http://www.cdc.gov/westnile/statsMaps/finalMapsData/data/2012WNVHumanInfectionsbyState.and http://www.cdc.gov/westnile/statsMaps/preliminaryMapsData/index.html). This has now been updated (pgs. 5 in text).

2. Page 4 “Background”. A recent reference that report the mortality rate (report likely as described by ref n. 8 – 2006 -) in the recent years should be added. This has now been added (pg. 5 in text).

3. Page 7 “Enrollment criteria”. The clinical criteria used to enroll the patients in the study has been subsequently supported by laboratory evidence of IgM detection in the CSF or in the serum (as an alternative the Authors state that “WNV disease had already been proven” but how this was proven should be disclosed). The CDC guidelines for the serological diagnosis of WNV human infection (see: http://www.cdc.gov/westnile/resources/pdfs/wnvGuidelines.pdf) clearly report that “IgM detection” is “a front-line assay” and that “Because the IgM and IgG ELISA tests can cross-react between flaviviruses (e.g., SLE, dengue, yellow fever, WN), they should be viewed as screening tests only.” As a consequence, a confirmatory test such as PRNT must be performed in due time for “a case to be considered as confirmed”. The Authors must discuss they results in the light
of these issue since there are many potentially “counfounding and related” flaviviruses that can hamper the findings of this study. *This has been addressed and see answer to Reviewer 1, Major Point 2*).

4. “Discussion”. No discussion about the results obtained with the therapeutic interventions….why do not to take out this part from the study? *This has been addressed and see answer to Reviewer 1, Major Point 1*).

Reviewer 3:

The manuscript by Hart J et al "West Nile virus encephalitis: Neurological manifestations and prospective longitudinal outcomes" is an interesting study on the clinical follow up (up to 90 days) of patients suffering from WNV encephalitis.

The study suffers however from two major issues that should be addressed before publication (see major compulsory revisions)

**Major Compulsory Revisions**

1. This is notably difficult to assess the adequacy of the methodology: 3 groups of patients were enrolled and given possible therapeutics, then considered as a unique group in the current study given the fact that no statistical difference was found between the groups. The problem here arises from the fact that no paper has been published on the comparison of the 3 therapeutic regimens and no data allows us to ascertain that the results from the current study are correct. The authors should be encouraged to publish their other manuscript first. *This has been addressed, see response to Reviewer 1, Major Point 1*).

2. Too few citations/references were given in the discussion section. The results do not seem to be compared and discussed to other studies in the field. The organization of the discussion section is not optimal: discuss the clinics (paragraphs 1, 2, 5, 3 death, 4 recovery), factors predicting the outcome (paragraphs 6), study limitations. *The Discussion has now been revised to address these issues (see text pgs. 23-24)*.

**Minor Essential Revisions**

3. Abstract p3, lines 13-16: reconsider revision of the sentence "represent/signal", "appears to predispose" *This has now been corrected as suggested (pgs. 3-4 in text)*.

4. Please use coherent figure in the text, abstract and tables

Abstract, line 19: "present in 9 at onset and 4 at study conclusion" *This has now been corrected as suggested (pgs. 3-4 in text)*.

Abstract, line 20: "11 continued... tremor" *This has now been corrected as suggested (pgs. 3-4 in text)*.

5. Introduction, p5, line 6 : if referring to case and death peaks, please cite the number of deaths in 2012 (286) or indicate the number of deaths in 2003 *This has now been updated with more current figures (pg. 5 in text)*.
6. Introduction, p5, line 9: 2012 figures are no more preliminary... and the introduction should be updated
   This has now been updated with more current figures (pg. 5 in text).

7. Introduction, p5, lines 10-11: if 80% individuals suffer from asymptomatic infection, there is no room
   for 20-30% mild infections. Please modify. This has been modified (text pg. 5).

8. Methods p 7 line 5: please cite the acronym (IVIG) here for easy reading of the manuscript This has
   now been corrected (pg. 9 in text).

9. The Methodology section could be shortened. It seems not clear if WNV encephalitis patients only
   were enrolled or if patients suffering from meningitis or polio-like syndrome were also considered for this
   study... If every patients suffering from WNV neuroinvasive disease were considered, the authors are
   invited to modify their title and abstract. We agree and this has now been done in the title and abstract
   (text pgs. 1-4).

10. Results, p13, lines 6-7: figures are not the same as the ones in table 1. Please correct. This has been
    corrected (text pgs. 13-14).

11. Results, p13, line 12-13: eleven patients ended their participation, but do not correspond to a total of
    7 deaths + 3 other reasons. This has been corrected (text pg.14).

12. Results section: the fact that 4 patients were normal at the beginning of the study is annoying. Did the
    corresponding patients really suffer from WNV neuroinvasive disease? Or should they be withdrawn
    from analysis? Symptomatic complaints and laboratory enrollment criteria showed that these individuals
    met criteria for WNV neuroinvasive disease. However, on the neurological tests used we were unable to
    discern a clear deficit for each of these individuals. We attribute this due to the insensitivity of our
    clinical measures to detect neurodysfunction in these cases.

13. Figures: Tables 3 and 4 could be simplified and gathered (also with table 2?). A figure on the
    correlation between variables (latest section of the result) would be valuable. We appreciate this
    comment, as we limited the number of tables for editorial considerations and given the other reviewers’
    comments, we are compelled to leave the table as is.

   Discretionary Revisions

14. Methods, p10, line 11: “determine the patient's level of” This is now corrected (text pg. 10).

Thank you and the Reviewers again for your careful comments and we hope you now find the manuscript
acceptable for publication.

Sincerely,

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