Author's response to reviews


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May 30, 2014

Editor-in-Chief

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Re: MS: 1554678346122581


Dear Professor Philippa Harris,

Thank you very much for your review of our submitted manuscript. Please find our responses to the expert reviewers’ comments. We appreciate each comment and have made a series of revisions that we believe have much improved the manuscript. Please do not hesitate to contact me if you have further questions or concerns.

Sincerely,

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Major Compulsory Revisions

1. Page 10

No description of statistical methods was noted in this section. This needs to be stated in this section.

- Thank you for noting this. We added the following sentence regarding the description of statistical methods.

“The incidence rates of AE were estimated as the case number (n) and proportion (%) and were compared using the chi-square or Fisher’s exact test to determine whether there was a difference between the treatment groups. Demographic characteristics, including sex, age, medical history, prior medication, and concomitant medication and vaccination, were compared between treatment groups using Student’s t-test or the Wilcoxon rank sum test for continuous variables and the chi-square or Fisher’s exact test for categorical variables. All statistical analyses were performed using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA).”

(Page 10, Line 22 – Page 11, Line 6; revised manuscript)

2. Page 30-33, 35, Tables 1-4, 6

The Fisher’s exact test was used for the comparison of numbers of subjects throughout the study. What was the reason why the authors did not use Chi-square test?

- Thank you very much for your inquiry. We made a mistake in describing the statistical method. The data were compared between treatment groups using the chi-square test as well as Fisher’s exact test for categorical variables. We revised the footnotes of Tables 1-4 and 6 as follows.

“*The p-values were calculated using the chi-square test for categorical variables and the Wilcoxon rank sum test for continuous variables,” (Page 32, Line 3-4; revised manuscript)

“*The p-value was calculated using the chi-square or Fisher’s exact test.” (Page 31, Line 4; Page 35, Line 6; Page 37, Line 3; revised manuscript)

“*Chi-square or Fisher’s exact test by the number of subjects” (Page 34, Line 3; revised manuscript)
3. Page 30, Table 1
The age was described as mean ± SD. Are these normally distributed?
- Thank you for this comment. The ages of the subjects were not normally distributed. We
changed these numbers for the median value and range as follows.

Table 1 Characteristics of the study subjects (n=204, safety population)

<table>
<thead>
<tr>
<th></th>
<th>KD-287 (n=102)</th>
<th>JEV-GCC (n=102)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male:Female</td>
<td>1.49:1</td>
<td>1:1</td>
<td>0.159</td>
</tr>
<tr>
<td>Age (months), median (range)</td>
<td>12.0 (12.0-22.0)</td>
<td>12.0 (12.0-18.0)</td>
<td>0.233</td>
</tr>
<tr>
<td>Medical history, n (%)</td>
<td>72 (70.59)</td>
<td>68 (66.67)</td>
<td>0.546</td>
</tr>
<tr>
<td>Prior medications, n (%)</td>
<td>40 (39.22)</td>
<td>40 (39.22)</td>
<td>1.000</td>
</tr>
<tr>
<td>Concomitant medications, n (%)</td>
<td>89 (87.25)</td>
<td>88 (86.27)</td>
<td>0.836</td>
</tr>
<tr>
<td>Concomitant vaccinations, n (%)</td>
<td>36 (35.29)</td>
<td>32 (31.37)</td>
<td>0.553</td>
</tr>
</tbody>
</table>

*The p-values were calculated using the chi-square test for categorical variables and the Wilcoxon rank sum test for continuous variables.

What is the reason why the rates of concomitant medications are so high?
- In this study, concomitant drugs were used in 87% of the investigational vaccine group and 86% of the control vaccine group.
Concomitant drug was monitored for approximately 14 weeks, that is, 6 weeks from enrollment to 4 weeks after the second vaccination and 8 weeks from 4 weeks before to 4 weeks after the third vaccination. Children aged 12-24 months frequently develop common colds, which are often complicated by acute otitis media (AOM) or acute bacterial sinusitis (ABS), especially in the day care environment. In South Korea, many children attend day care centers, and prescriptions are readily available from the health care system. In fact, cold preparations were prescribed to 72 (71%) subjects in the KD-287 group and 70 (69%) in the JEV-GCC group during the monitored period. Antibiotics were prescribed to 66 (65%) subjects in the KD-287 group and 68 (67%) subjects in the JEV-GCC group. However, the concomitant use of each drug was not different between the two groups, and we assume that the use of concomitant drugs did not influence the major outcomes of the study. We added the following sentences regarding the high rates of concomitant medications.

"The rates of concomitant medications were high because the subjects might possibly
contract a common cold or bacterial respiratory infection such as otitis media and sinusitis during the relatively long monitoring period of 14 weeks. Cold preparations and antibiotics were prescribed to 72 (71%) and 66 (65%) subjects in the KD-287 group, respectively and 70 (69%) and 68 (67%) subjects in the JEV-GCC group, respectively during the study (data not shown).” (Page 12, Line 4-9; revised manuscript)

Minor Essential Revisions

1. Page 8, Line 16
Please describe in details regarding “reported passively during a telephone interview”. Did the authors call for all guardians or parents after the vaccination to interview?
- We appreciate the reviewer’s comment. We called all guardians or parents at 7 days and 6 months after the booster vaccination. We changed the following sentence.
“All serious AEs (SAEs) between day 0 and 6 months after the booster vaccination were reported actively by parents/guardians or passively during a telephone interview.” (Page 8, Line 15-17, original manuscript)

“All serious AEs (SAEs) between day 0 and 6 months after the booster vaccination were reported actively by parents/guardians or passively during the two telephone interviews at seven days and six months after the booster vaccination.” (Page 8, Line 18-20; revised manuscript)

2. Page 10, Line 15
What does PP stand for?
- “PP” means “Per-protocol”. We described this full name on Page 6 (Line 22) of the original manuscript.

3. Figure 1
First box on right, reason
Not received any vaccine should read “Received any vaccine”.
The JEV-GCC (N=102) should read JEV-GCC (N=102).
- Thank you for noting this. In the first box on the right, one subject in the KD-287 group was
excluded due to withdrawal immediately after randomization. Because the subject did not receive a vaccine, the subject was not included in the safety population. To state this fact more clearly, we changed the following phrase.

“Not received any vaccine” (Figure 1; original manuscript)

“Not received any vaccine (withdrawal just after randomization)” (Figure 1; revised manuscript)

4. Figure 3

It is very difficult to distinguish from each blue and red line. Please describe or add arrows to clarify.

- We appreciate the reviewer’s comment. We revised Figure 3 and added the following sentences to differentiate the lines more clearly.

“Lines drawn with a character “x”, open circle (o), and rhombus (◆) indicate the antibody titers after the second vaccination, and before and after the third vaccination, respectively, in both groups. Lines for the JEV-GCC and KD-287 groups are drawn in blue and red, respectively.” (Page 30, Line 16-20; revised manuscript)

Discretionary Revisions

1. Page 8, Line 3

A regular interval for inactivated vaccine for primary series is usually 4 weeks.
What is the reason this was set for an interval of 2 weeks?
- We appreciate the reviewers’ question. However, a regular interval for an inactivated vaccine (JEV-GCC) for a primary series is set at 1-2 weeks in Korea. Because this interventional vaccine is intended to replace the previous JEV-GCC vaccine, the interval for the primary series was set at 2 weeks.

2. Page 21 Line 14, Page 32, Table 3
Although the authors described nasopharyngitis was not directly related to vaccine, what is the reason why the incidence of nasopharyngitis is so high up to 80%?
- Thank you very much for noting this. We mistakenly represented some incorrect data in Table 3. The incidence rates of nasopharyngitis were 60% and 55% in the KD-287 and JEV-GCC groups, respectively. We revised Table 3 as follows.

Table 3 Incidence of frequently (≥10%) reported adverse events (n=204, safety population)

<table>
<thead>
<tr>
<th></th>
<th>KD-287 (n=102)</th>
<th>JEV-GCC (n=102)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Subjects, n (%)</td>
<td>Events, n</td>
<td>Subjects, n (%)</td>
</tr>
<tr>
<td>Solicited</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythema</td>
<td>27 (26.47)</td>
<td>40</td>
<td>32 (31.37)</td>
</tr>
<tr>
<td>Pain</td>
<td>30 (29.41)</td>
<td>41</td>
<td>22 (21.57)</td>
</tr>
<tr>
<td>Swelling</td>
<td>16 (15.69)</td>
<td>21</td>
<td>19 (18.63)</td>
</tr>
<tr>
<td>Fever</td>
<td>52 (50.98)</td>
<td>66</td>
<td>41 (40.20)</td>
</tr>
<tr>
<td>Crying</td>
<td>47 (46.08)</td>
<td>68</td>
<td>40 (39.22)</td>
</tr>
<tr>
<td>Irritability</td>
<td>26 (25.49)</td>
<td>33</td>
<td>26 (25.49)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>14 (13.73)</td>
<td>18</td>
<td>17 (16.67)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>30 (29.41)</td>
<td>39</td>
<td>25 (24.51)</td>
</tr>
<tr>
<td>Decreased appetite</td>
<td>24 (23.53)</td>
<td>27</td>
<td>23 (22.55)</td>
</tr>
<tr>
<td>Somnolence</td>
<td>23 (22.55)</td>
<td>31</td>
<td>31 (30.39)</td>
</tr>
<tr>
<td>Unsolicited</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasopharyngitis</td>
<td>61 (59.80)</td>
<td>115</td>
<td>56 (54.90)</td>
</tr>
<tr>
<td>Otitis media</td>
<td>16 (15.69)</td>
<td>21</td>
<td>9 (8.82)</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>10 (9.8)</td>
<td>12</td>
<td>14 (13.73)</td>
</tr>
<tr>
<td>Pharyngitis</td>
<td>7 (6.86)</td>
<td>8</td>
<td>11 (10.78)</td>
</tr>
<tr>
<td>Gastroenteritis</td>
<td>2 (1.96)</td>
<td>2</td>
<td>11 (10.78)</td>
</tr>
</tbody>
</table>

*Chi-square or Fisher’s exact test by the number of subjects
The solicited and unsolicited AEs were collected for 4 weeks after each vaccination.

3. Page 33, Table 4

What is the definition of pneumonia? Why the incidence of pneumonia is so high in both populations?

- The diagnosis of pneumonia was made by individual physicians who had admitted the subjects of this study. It appears that bronchiolitis and asthmatic bronchitis might be considered to be pneumonia without definitive diagnostic criteria in these settings. Furthermore, because the study subjects were primarily patients that frequently visited the each site, there could be a selection bias for medical attention and risk factors. However, we considered these factors to be unrelated to the results of this randomized controlled study.
1. Background, paragraph 3.

Although this study evaluated non-inferiority for an established immunologic correlate of protection which may be used for licensure, I believe it should be referred to as a phase 2 study with an outcome of safety and immunogenicity rather than a phase 3 study with an outcome of efficacy or effectiveness.

- We appreciate the reviewer’s comment. As indicated by the reviewer, this study was conducted to evaluate the safety and immunogenicity rather than the efficacy or effectiveness of an investigational vaccine.

However, the clinical evaluation of new JE vaccines for efficacy is complicated by the low incidence of JE and the availability of an internationally registered product. Under these circumstances, a placebo-controlled trial would be difficult to justify on ethical grounds, and a comparative trial between licensed and new vaccines using the endpoint of prevention of clinical illness would require impractically large sample sizes. The neutralizing antibody for JE virus after vaccination is a well-known surrogate marker of protection. The non-inferiority results of the test vaccine with respect to the surrogate marker suggest at least an equivalent clinical efficacy (Tauber E, Lancet 2007, 370: 1847-1853). Hence, the WHO recommends that the efficacy of a new JE vaccine is demonstrated by showing equivalent neutralizing antibody titers compared to the known protective levels produced by a licensed vaccine (Hombach J. Vaccine 2005, 23: 5205-5211).

Based on these limitations, many clinical studies similar to the current study have been conducted using the design of a phase III trial; some examples are listed below.

- Phase III clinical trials comparing the immunogenicity and safety of Vero cell-derived Japanese encephalitis vaccine ENCEVAC(R) with those of mouse brain-derived vaccine using

Therefore, we would like to retain ‘phase III trial’ in the title.

2. Results, Study population, paragraph 1.
Why the three subjects who were enrolled and randomized to a study group but did not have any immunogenicity data were excluded from the ITT analysis.
- Thank you for the comment. This study was conducted primarily to identify the immunogenicity of the vaccine. The three subjects who did not have immunogenicity data should be considered missing subjects, and we thus excluded these subjects from the intention-to-treat (ITT) group.

3. Discussion, paragraph 5.
IC51 is licensed for use in adults and children aged 2 months or older in Europe and the United States. The vaccine available in India (JEEV) is very similar to IC51 but is manufactured by Biological E using technology transferred from Intercell.
- Thank you very much for noting this. We changed the following sentences.

“IC51 is available in the United States, Europe, Canada, Switzerland, and Australia, and more recently in India. IC51 has been licensed and indicated for prevention of JE in individuals aged ≥17 years [21]. Recently, the US Advisory Committee on Immunization Practices (ACIP) extended the existing recommendations for use of IC51 to include children aged 2 months to 16 years [22].” (Page 16, Line 16-20; original manuscript)

“IC51 is available in 38 countries in North America, Europe, Asia, and Oceania. IC51 has been licensed and indicated for prevention of JE in individuals aged ≥2 months in the United States and Europe. Recently, a JE-VC similar to IC51 became available in India and is
manufactured by Biological E using technology transferred from Intercell [21,22].”
(Page 17, Line 8-12; revised manuscript)

4. Discussion, paragraph 7.
The two-fold difference in GMTs between IC51 and JE-MB was with titers against the homologous strain for IC51 and heterologous strain for JE-MB.
- Thank you for this comment. We revised the following passage.
“In addition to having a favorable safety and tolerability profile, the SCR induced by two IC51 (SA 14-14-2 strain) doses (98%) was demonstrated to be non-inferior to the SCR induced by three doses of a JE-MB (Nakayama strain) (95%), and the GMT after IC51 vaccination was 2-fold higher than that after JE-MB vaccination [8, 9].” (Page 17, Line 22 – Page 18, Line 3, Original manuscript)

“In addition to having a favorable safety and tolerability profile, the SCR induced by two IC51 (SA 14-14-2 strain) doses (98%) was demonstrated to be non-inferior to the SCR induced by three doses of a JE-MB (Nakayama strain) (95%). Furthermore, the GMT after IC51 vaccination was 2-fold higher than that after JE-MB vaccination [8, 9], although the GMTs were evaluated against the homologous strain for IC51 and the heterologous strain for JE-MB.” (Page 18, Line 14-19; revised manuscript)

5. Discussion, paragraph 7.
Boosting with a single dose of IC51 following 3 or more doses of JE-MB was also reported by Woolpert et al. Vaccine 2012;30:3090.
- Thank you for the comment. We changed the following sentence and added the new reference 28.
“Recently, Erra et al. found that a single dose of IC51 effectively boosted immunity in travelers primed with a JE-MB (Nakayama strain) [14].” (Page 18, Line 7-9, Original manuscript)

“Recently, two studies showed that a single dose of IC51 effectively boosted immunity in adults primed with a JE-MB (Nakayama strain) [14, 28].” (Page 19, Line 1-2; revised manuscript)
6. Table 1.
Please clarify what is included in medical history, prior medications, concomitant medications, and concomitant vaccinations.
- We added the following sentences to the footnote of Table 1 for clarification.
  “Medical history included all major medical problems such as hospital admission, surgery, and long-term medication.
  Prior medications included all medications administered to the subject within 4 weeks of vaccination.
  Concomitant medications and vaccinations included all medications and vaccinations administered to the subjects for 6 weeks from the first vaccination to 4 weeks after the 2nd vaccination and during the 8 weeks from 4 weeks before to 4 weeks after the 3rd vaccination.” (Page 32, Line 5-10; revised manuscript)

7. Table 2.
Please confirm that the $p$-value refers to the difference in proportions of subjects with adverse events (i.e., as opposed to number of events).
- We changed the following sentence for clarification.
  “The $p$-value was calculated using the Fisher’s exact test.” (Page 31, Line 4; original manuscript)
  “The $p$-value refers to the difference in the proportions of subjects with adverse events and was calculated using the chi-square or Fisher’s exact test.” (Page 33, Line 4-5; revised manuscript)

8. Table 3.
Were unsolicited adverse events also collected for the 7 days after each vaccination or are
these for the entire study period?

- Thank you for this comment. Solicited and unsolicited adverse events except SAEs were collected for 4 weeks after each vaccination; the data were recorded daily for the first 7 days and were incidentally recorded for the next 21 days on a diary card. We added the following sentence.

“The solicited and unsolicited AEs were collected for 4 weeks after each vaccination.” (Page 34, Line 5; revised manuscript)

What data are provided or highlighted in these figures that are not available in the tables?

- We appreciate the reviewer’s comment. As the reviewer noted, Figure 3 and Figure 4 provide the data included in Table 6. However, Figure 3 highlights the difference in the distribution of neutralizing antibody levels against JEV in the two groups. Figure 4 highlights the higher GMTs before the third vaccination in the KD-287 group compared to after the second vaccination for both tested strains; the GMTs were enhanced in both groups after the third vaccination. Table 3 provides the p-values and 95% CIs (especially the value of the lower limit, which was used for identifying fulfillment of non-inferiority of the investigational vaccine to the control vaccine) as well as the GMT values for both groups.

Nevertheless, we agree with the reviewer’s opinion that the data in Figures 3 and 4 are largely repeated in Table 6. Hence, we decided to move Table 6 to the supplementary material (Table S1).