Author’s response to reviews

Title: A randomized, controlled non-inferiority trial comparing H1N1 2009 pandemic vaccine antigen, with and without AS03 adjuvant system, co-administered or sequentially administered with an inactivated trivalent seasonal influenza vaccine

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Author’s response to reviews: see over
14 August 2012

Philippa Harris  
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Re: MS: 1674755986462813

A randomized, controlled non-inferiority trial comparing H1N1 2009 pandemic vaccine antigen, with and without AS03 adjuvant system, co-administered or sequentially administered with an inactivated trivalent seasonal influenza vaccine. Joanne M Langley, Louise Frenette, Laurence Chu, Shelly McNeil, Scott A. Halperin, Ping Li and David W Vaughn

Dear Ms. Harris,

Thank you for decision to re-review our work. Please find below our responses to the two reviewer comments, and the revised manuscript.

Reviewer 2. Version: 2 Date: 4 June 2012 Reviewer: Zol Vaj

The authors responded to some issues raised by the reviewers and argued with others. Importantly, they have made some improvements regarding CONSORT compliance. They have also included at least some weaknesses and limitations of their study.

Specific comments:

1. **Comment:** The authors state and emphasize repeatedly that they had worked with "only adults less than 40 years of age without a history of receiving TIV to work with a more influenza naive population" - Being "influenza naive" is not defined by age or a previous history of TIV administration, but by pre vaccination HA titers. It appears that up to 25% of the subjects were seroprotected at baseline already. Thus, I don't think the points on "influenza naive" can be made.

   **Response:** In the discussion this point is made (page 12). We have rechecked the document to ensure it reads “TIV naïve”. The point about up to 25% of the population being seroprotected at baseline to the pandemic strain is already made in the discussion so we have not changed this. We have reworded the section as follows:

   “There are several limitations to our study. The sample included TIV-naïve subjects, that is adults less than 40 years of age without a history of receiving TIV or the pandemic vaccine, in order to increase the likelihood that subjects had little or no prior exposure to influenza, reducing age-based immunogenicity-variation and allowing for a smaller sample size. About 10.5 to 25% of subjects were not naïve to the pandemic strain. Thus, these results may not be generalizable to older adults or those with prior influenza vaccine exposure.”

2. **Comment:** Please provide p values for safety and reactogenicity comparisons.

   **Response:**
Currently safety and reactogenicity comparisons are provided in Figures 2 and 3, in which a histogram with 95% confidence intervals for each outcome is shown. No statistical comparisons were performed between groups. The presentation of point estimates and confidence intervals for the multiple injection site and systemic adverse events that were monitored allows the reader to see the range of the possible frequency of adverse events. Where confidence intervals are overlapping one can conclude that there was similar frequency across groups.

We have revised the title of the Figure where these results are shown to read “Incidence and 95% CI of solicited local and general symptoms recorded during the 7-day post-vaccination follow-up period (Total vaccinated cohort)”

We have added to the statistics section, as follows:

“No statistical comparisons were performed between groups for safety and reactogenicity”

3. **Comment:** Were the sera tested independently to confirm the results? There are known issues with the high inter-laboratory variation of HA assays.

**Response:**
HI samples were tested in duplicate at one laboratory. The following has been added in the manuscript methods section “Serum samples were collected before vaccination, 21 days after each vaccine dose and six months after the first vaccine dose (Days 0, 21, 42, 63 and 182) and tested in duplicate for haemagglutination inhibition (HI) titres using a validated assay [cut-off: \( \geq 1:10 \)] with chicken erythrocytes as previously described,[13] at GSK laboratories. (Page 15)

We acknowledge that the HI assay (like other bioassays) is a variable assay, however, a quality system is in place at GSK laboratory to ensure the quality of lab testing. The assay is fully validated and the co-efficient of variation tested during the validation testing was measured at 27.3% for the H1N1 HI assay.

In case the titres calculated for each duplicate are divergent of more than 2 titre steps, sample testing was repeated. The assay is validated when control sera titers tested in the same run are in a defined range of variation when compared to reference titre. GSK also participated in the H1N1 international standardisation exercise organized by the reference centers (Wagner et al., Vaccine 30(27): 4113-22, 2012). HI testing performed on the same samples at a reference laboratory and GSK HI testing laboratory presented a very good qualitative correlation and a good quantitative correlation for sera. We hope this information will satisfy the reviewers regarding the quality of data generated at GSK HI testing laboratory.

4. **Comment:** Can the authors provide microneutralization results for immunogenicity?

**Response:**
Microneutralization testing was not done.

5. **Comment:** Discussion section: "Grade 3 pain inject(sic) site pain" - Rephrase this.

**Response:**
This sentence has been reworded to: “Grade 3 injection site pain was uncommon.”

6. **Comment:** "Neither the subjects or(sic) study personnel evaluating the safety and immunogenicity were aware of vaccine assignments" - how was blinding maintained if the adjuvanted and non-adjuvanted vaccines had a different appearance?

**Response:**
Different study personnel were responsible for administering vaccine. A description is provided in the previous paragraph to this sentence (page 17), to which additional clarification has been added (see italics):

“Patients were enrolled by trained study personnel. Once eligibility was confirmed, specific unblinded study personnel were responsible for determining vaccine allocation as assigned by the internet based randomization system at each vaccination visit, vaccine preparation, and administration. The unblinded staff accessed the randomization system on the internet and provided the age and identification number of the participant. The randomization system assigned a treatment number which mapped to a vial number corresponding to supplies at that
study site. Vaccine reconstitution by the unblinded nurse was done in a secure room, and then the individual dose carried to the participant’s room on a tray covered by an opaque cloth. The unblinded personnel had no other role in the study. Neither the subjects or blinded study personnel evaluating the safety and immunogenicity endpoints were aware of vaccine assignment until the data analysis was completed.”

7. **Comment:** "Study vaccine not administered N=260" - please provide reasons for this.

**Response:**

871 subjects were enrolled and then screened. The screening consisted of blood tests for liver function test and a complete blood count (CBC). Those whose laboratory tests did not fall in the reference ranges for normal results did not participate further in the study.

**Reviewer 1:**

1. **Comment:** The manuscript has been significantly improved and there is just one minor comment. The authors asked suggestions on how to present the information of the study sites. Based on the information in the response to my Comment 4, you could create a new Table 1 like below:

<table>
<thead>
<tr>
<th>Location of study site</th>
<th>Number of participant (611 in total)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Country</strong></td>
<td><strong>Province or State</strong></td>
</tr>
<tr>
<td>Canada</td>
<td>Quebec</td>
</tr>
<tr>
<td></td>
<td>Nova Scotia</td>
</tr>
<tr>
<td></td>
<td>Montreal</td>
</tr>
<tr>
<td>USA</td>
<td>Austin, Texas</td>
</tr>
<tr>
<td></td>
<td>Raleigh, North Carolina</td>
</tr>
<tr>
<td></td>
<td>Stockbridge, Georgia</td>
</tr>
<tr>
<td></td>
<td>Fort Wort, Texas</td>
</tr>
</tbody>
</table>

Then on page 4, the first paragraph under Results section, change “Of the 871 subjects enrolled and screened, 611 subjects were vaccinated;” into “Of the 871 subjects enrolled and screened, 611 subjects were vaccinated (Table 1). Also, on Page 12, the first sentence under “Trial design” in Methods section, change it from “conducted at four sites in the United States and three in Canada.” to “conducted at three sites in Canada and four in the United States (Table 1).”

**Response:**

We have created a table and adjusted numbering accordingly.

<table>
<thead>
<tr>
<th>Country</th>
<th>Province or State</th>
<th>Number of sites</th>
<th>Participants enrolled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canada</td>
<td>Quebec</td>
<td>2</td>
<td>225</td>
</tr>
<tr>
<td></td>
<td>Nova Scotia</td>
<td>1</td>
<td>61</td>
</tr>
<tr>
<td>USA</td>
<td>Texas</td>
<td>2</td>
<td>195</td>
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<tr>
<td></td>
<td>North Carolina</td>
<td>1</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Georgia</td>
<td>1</td>
<td>93</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>7</td>
<td>611</td>
</tr>
</tbody>
</table>

Thank you for your attention to these revisions. If you have any questions please do not hesitate to contact me.

Sincerely,

Joanne Langley MD MSc FRCPC
Corresponding Author