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
30 March 2012

BMC Infectious Diseases

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 Halperin, Ping Li and David W Vaughn BMC Infectious Diseases

Dear Sir/Madam,

Thank you for your review of our manuscript, and your interest in reconsidering it following revision. Enclosed please find a revised paper. This letter outlines the point-by-point reviewer comments and our revisions and responses.

If you have any further questions please do not hesitate to contact me.

Sincerely.

Joanne Langley

Reviewer 1

1. The topic is of interest, although several publications have addressed this issue before.

Response

We are not aware of any other studies that assess concurrent administration of TIV and pandemic vaccines in TIV-naive populations.

2. One of the major weaknesses of this study is the lack of any patients[sic] over 40 years of age.

Response

We recruited young adults without a history of influenza vaccination to assess immune responses in a relatively immunologically naive population (as naive as possible as most or all would have previously experienced influenza virus infections) to maximize the influence of study-provided TIV versus placebo. The narrow age range was also intended to reduce age-based immunogenicity variation (GMTs reduced above 40 years
of age in some GSK pre-pandemic vaccines studies) to allow for a smaller sample size. It is true that the limitation of participants to this age group means that the results may not be generalizeable to older age groups. We have added this idea to the discussion. It is true that the limitation of participants to this age group means that the results may not be generalizeable to older age groups. We have added this idea to the discussion. In the “Study Vaccine” section, an error message is seen in the text: “error, reference not found”.

Response

All reference citations have been rechecked and notation corrected.

4. I see no allocation concealment mechanism.

Response

Please see the response to question 5, below.

5. It is not reported who generated the allocation sequence, who enrolled participants, and who assigned participants to interventions?

Response

The methods are rewritten to consolidate the information on randomisation, as per the CONSORT checklist. This section now reads:

“Patients were randomized 1:1:1:1:1:1 to the six study groups. The randomization was performed by the sponsor using MATEX, a program develop for use in SAS (Cary, NC, USA) by GSK Biologicals, Rixensart, which incorporated a minimization algorithm. Study supplies were distributed to each study center by blocks containing all vaccine supplies. Patients were enrolled by trained study personnel. Once eligibility was confirmed, specific unblinded study personnel, who had no other role in the study, 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 person 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 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.”
Neither the subjects or study personnel evaluating the safety and immunogenicity endpoints were aware of vaccine assignment until the data analysis was completed.”

6. *Were [sic] the adjuvant really mixed with the vaccine on the day of vaccination by a nurse? This does not seem to be GMP compliant. How was the quality and accuracy assured? I see that the ratio was 1:1 but what volumes were used? Was this an FDA approved method?*

**Response**

The vaccine used in this study or similar product with vaccine antigen produced in Germany) was employed in national campaigns in Canada, in several European countries, and in other counties using this method. The study was approved by the FDA and funded by the US Department of Health and Human Services. The approving regulators are listed on the NCT register page, which can be found through the NCT number that was provided on the title page.

7. *Was the mixed product, containing the adjuvant, similar in appearance of the non-adjuvanted vaccine? How about the appearance of the placebo?*

**Response**

“The monovalent pandemic vaccine without adjuvant, and the TIV, were translucent to whitish suspensions. The AS03 is a white liquid, and when mixed with influenza antigen is a whitish emulsion. “

8. *The TIV used in the trial is not with the approved strains for the 2009-10 season, which were the A/Brisbane/59/2007 (H1N1)-like virus, the A/Brisbane/10/2007 (H3N2)-like virus and the B/Brisbane/60/2008-like virus. Why did the investigators use a vaccine that was not approved for the season when the study was conducted?*
This is incorrect. As can be seen on the FDA website, all strains used in the vaccine were recommended by the WHO for the Northern hemisphere, and by North American regulatory authorities. The reviewer may not know that the recommended strain A/Brisbane/10/2007 (H3N2-) and A/Uruguay/716/2007 were current vaccine viruses and either could be used. This information can be found at:

<http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Post-MarketActivities/LotReleases/ucm162050.htm

9. *The subject numbers in each group (85-92) seem to be too low to prove non-inferiority.*

**Response**

More detail is now added to section on sample size, which now reads:

“A sample size of 600 subjects was estimated to provide a power of 88.35% to evaluate each of the co-primary objectives. The unevaluable subject rate was estimated at ≤5%, the Log Standard Deviation for the GMT assumed to be 0.6, and the type 1 error 0.025. The two co-primary objectives were evaluated in parallel, in terms of GMT ratio adjusted by pre-vaccination antibody titre. The study objective was considered met if one of the co-primary objectives was met. Hence, 97.5% confidence intervals (CIs) were used for the primary objective evaluation.”

**Results**

10. The baseline demographic and clinical characteristics should be reported, preferrably [sic] in a table.

**Response**

A table has been added, as below:

**TABLE 1. Summary of demographic characteristics (ATP cohort for immunogenicity)**
<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Parameters or Categories</th>
<th>A N = 92</th>
<th>B N = 86</th>
<th>C N = 92</th>
<th>D N = 89</th>
<th>E N = 91</th>
<th>F N = 91</th>
<th>Total N = 541</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Mean</td>
<td>29.6 -</td>
<td>28.8 -</td>
<td>28.0 -</td>
<td>29.3 -</td>
<td>29.6 -</td>
<td>28.5 -</td>
<td>29.0 -</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>6.30 -</td>
<td>6.46 -</td>
<td>6.41 -</td>
<td>6.21 -</td>
<td>6.45 -</td>
<td>6.19 -</td>
<td>6.34 -</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>29.5 -</td>
<td>28.0 -</td>
<td>27.0 -</td>
<td>29.0 -</td>
<td>29.0 -</td>
<td>27.0 -</td>
<td>28.0 -</td>
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<td></td>
<td>Maximum</td>
<td>40 -</td>
<td>40 -</td>
<td>40 -</td>
<td>40 -</td>
<td>40 -</td>
<td>40 -</td>
<td>40 -</td>
</tr>
<tr>
<td>Gender</td>
<td>Female</td>
<td>47</td>
<td>51.1</td>
<td>49</td>
<td>57.0</td>
<td>43</td>
<td>48.3</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>45</td>
<td>48.9</td>
<td>37</td>
<td>43.0</td>
<td>42</td>
<td>45.7</td>
<td>34</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>American hispanic or latino</td>
<td>11</td>
<td>12.0</td>
<td>9</td>
<td>10.5</td>
<td>7</td>
<td>7.6</td>
<td>5</td>
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<tr>
<td></td>
<td>Not american hispanic or latino</td>
<td>81</td>
<td>88.0</td>
<td>77</td>
<td>89.5</td>
<td>85</td>
<td>92.4</td>
<td>84</td>
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<td>Geographic Ancestry</td>
<td>African heritage / african American</td>
<td>14</td>
<td>15.2</td>
<td>11</td>
<td>12.8</td>
<td>14</td>
<td>15.2</td>
<td>13</td>
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<td></td>
<td>American indian or alaskan native</td>
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<td>1.1</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Asian - central/south asian heritage</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Asian - east asian heritage</td>
<td>0</td>
<td>0.0</td>
<td>1</td>
<td>1.2</td>
<td>1</td>
<td>1.1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Asian -</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>Characteristics</td>
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<td>A N = 92</td>
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<td>C N = 92</td>
<td>D N = 89</td>
<td>E N = 91</td>
<td>F N = 91</td>
<td>Total N = 541</td>
</tr>
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</tr>
<tr>
<td></td>
<td>Value or n</td>
<td>%</td>
<td>Value or n</td>
<td>%</td>
<td>Value or n</td>
<td>%</td>
<td>Value or n</td>
<td>%</td>
</tr>
<tr>
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<td>Heritage</td>
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<td>0 0.0</td>
<td>0 0.0</td>
<td>0 0.0</td>
<td>2 2.2</td>
<td>3 0.6</td>
</tr>
<tr>
<td>Asian</td>
<td>- South east asian heritage</td>
<td>0 0.0</td>
<td>0 0.0</td>
<td>0 3.3</td>
<td>0 0.0</td>
<td>0 0.0</td>
<td>0 0.0</td>
<td>3 0.6</td>
</tr>
<tr>
<td>Native</td>
<td>Hawaiian or other pacific islander</td>
<td>74 80.4</td>
<td>72 83.7</td>
<td>73 79.3</td>
<td>75 84.3</td>
<td>78 85.7</td>
<td>70 76.9</td>
<td>442 81.7</td>
</tr>
<tr>
<td>White</td>
<td>- Arabic / north african heritage</td>
<td>2 2.2</td>
<td>1 1.2</td>
<td>1 1.1</td>
<td>0 0.0</td>
<td>2 2.2</td>
<td>2 2.2</td>
<td>8 1.5</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A = TIV+PI d0, 15 mcg d21,42
B = TIV+PI d0, 3.75 mcg Adj d21,42
C = 15 mcg + TIV d0, 15 mcg d21, PI d42
D = 3.75 mcg Adj + TIV d0, 3.75 mcg Adj d21, PI d42
E = 15 mcg + PI d0, 15 mcg d21, TIV d42
F = 3.75 mcg Adj + PI d0, 3.75 mcg Adj d21, TIV d42

N = total number of subjects

n/% = number / percentage of subjects in a given category

Value = value of the considered parameter

SD = standard deviation
11. Reasons for not vaccinating more than 250 enrolled subjects are not provided.

Response

611 subjects were vaccinated. The number 250 does not appear in the manuscript, so we are uncertain what the reviewer is referring to. The patients who were enrolled but not vaccinated were screen failures.

12. The rate and severity of reactions seem to be excessive with the adjuvanted formula: >90% for reactions and over one third of the subjects required medical visits due to reactions - this vaccine cannot be described as "well tolerated".

Response

The manuscript does not report that one third of subjects required medical visits due to reactions. Perhaps the reviewer is confusing this with the report of unsolicited reactions (any health event occurring after vaccine to day 84), which are not the same as solicited reactions (injection site reactions or systemic reactions) in the week following vaccination. These unsolicited reactions were not different between groups and <5% of severe (Grade 3) events were considered causally related to vaccination. The frequency of injection site pain without swelling or redness is clearly identified as an important adverse event in the text, and the phrase “well-tolerated” removed.

Reviewer 2

The manuscript by Langley and colleagues presents an RCT on immune response comparing AS03-adjuvanted and non-adjuvanted Influenza A(H1N1)pdm09 vaccine with co- or sequentially given with TIVs. This is well-focused scope that certainly benefits from a RCT with comprehensive study design. The following points, however, should be addressed before it would be accepted for publication in BMC Infectious Diseases.

Major Compulsory Revisions

1. An important point of note is that CONSORT statement (http://www.consort-statement.org/consort-statement/) has been the international standard for the reporting of
an RCT. Although the present manuscript adheres to this standard to some extent, fully following the CONSORT statement would help better deliver the message particularly for a RCT comprehensive like this.

Response

The methods and results sections were reformatted to follow the CONSORT.

2. There is a lack of Limitation section in Discussion.

Response:

A limitation section is added.

Minor Essential Revisions

1. Try to use the new terminology for pandemic 2009 A (H1N1) virus (now is ?Influenza A(H1N1)pdm09, (www.nejm.org/doi/full/10.1056/NEJMc1111078) in the whole manuscript.

Response

The url provided by the reviewer is a link to a Letter to the Editor of NEJM reporting an oseltamivir resistant influenza virus. The nomenclature suggested by the reviewer was indeed approved by the WHO but is not commonly used, however we have substituted this nomenclature in the paper.

Background

2. Page 5, the 1st paragraph: the 2nd and 3rd sentences should be removed from Background and their contents should be reflected in Results instead. 3. Inset an Objective section in Background. The authors stated their objectives starting from the 2nd paragraph on Page 17 until the 1st paragraph on Page 18 in Methods, which is not an appropriate place. Remove them to the end of Background.

Response
It is a requirement of BMC Infect Dis that the background “section should end with a brief statement of what is being reported in the article.” To follow the reviewers advice would make the article non-compliant with the instructions to authors.

Further, the objectives are at the beginning of that paragraph. We have not revised this section, pending editorial office guidance.

Results and Discussion

3. Page 5, Line 11: the 1st sentence, ?The study was conducted...? should be moved to Methods (Page 13, Line 6-7, right after ?......and three in Canada?) 5. Page 5, Line 11, the sentence of ?Of the 871 ....?: better to say ?Of the 871 subjects approached and screened, 611 of them were enrolled and vaccinated....?. I doubt if appropriate to say ?enrolled? if a person was a screening failure.

Response

The dates over which the study was conducted cannot be planned prior to initiation of the study, and we argue therefore do not fit in methods. 871 subjects were enrolled and screened, and 611 vaccinated, so we have not changed this wording.

4. The Demographics section on Page 5: please give more information on the 4 study sites, i.e. what cities were they? How many participants from each site?

Response

There are 7 study centers (4 USA, 3 Canada). Below is the info requested by the reviewer. Kindly suggest on how to present this in the manuscript.

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Address</th>
<th>Participants (# enrolled)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Louise Frenette</td>
<td>95 Camirand Street Suite 130, Sherbrooke, Quebec, Canada</td>
<td>163</td>
</tr>
<tr>
<td>Joanne Langley</td>
<td>5850 University Avenue, Goldbloom Research Pavilion, 4th Floor, Halifax, Nova Scotia, Canada</td>
<td>61</td>
</tr>
<tr>
<td>Pierre Lanouette</td>
<td>1851 Sherbrooke Street East Suite 502 Montreal, Canada</td>
<td>62</td>
</tr>
</tbody>
</table>
5. It would be better to move the "CHMP and CBER criteria? section (starting from Line 6 to 17 on Page 8) to Page 5 and just under the heading of "Immunogenicity".

Response

The document has been reformatted to better conform to the CONSORT checklist; hopefully these statistically derived outcomes are appropriate placed in the revised paper.

6. I wonder if slightly more comment is deserved on the controversy of this topic (whether TIV would confer cross-reacting antibodies to Influenza A[H1N1]pdm09 virus or cross-protection to Influenza A[H1N1]pdm09 disease). A serology study by Hancock(Hancock et al., 2009) reported that TIV did not seem to confer cross-reacting antibodies against Influenza A(H1N1)pdm09. Some other studies, i.e. Xia(Xie et al., 2011) and Lee(Lee et al., 2010) (Lee was already cited by the authors on Page 11), provided opposite results. It may worth commenting on the substantial heterogeneities of these results, for example, different population samples, study designs etc. Adding to the 3rd paragraph on Page 11, the argument in this manuscript would become even stronger. At the vaccine effectiveness/efficacy level, the controversy also exists, probably to the bigger extent (i.e. the Mexican study(Garcia-Garcia et al., 2009), the Canadian data(Skowronsli et al., 2010) et al.). How does this RCT add to the scope of this topic although it is an immunogenicity study? This inconsistency or consistency between immunogenicity and efficacy/effectiveness would seem to be worthy of comment.

Response

The pandemic raised a number of issues regarding immune responses to seasonal influenza and drifted and shifted strains. This study does not attempt to address either of two particularly interesting concepts the reviewer describes: 1) the observation made between the first and second wave of the 2009 pandemic in a case-control study that TIV vaccination in the previous season was associated with a visit to a family doctor’s office for influenza due to the pandemic strain, or 2) whether TIV confers cross-reacting antibodies to the pandemic virus.
In our study TIV was given concurrently or sequentially with monovalent pandemic virus vaccine in TIV- naive adults in order to determine if one or the other schedule had preferable immunogenicity or reactogenicity.

7. Page 14, Line 17: correct the reference (Error! Reference source not found.)

Response

All references have been checked and corrected.

Thank you again for assistance with the preparation of this work.

Best wishes,

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